

# A Microscopic Study on the Egg Envelope of an Endemic Korean Fish, *Coreoleuciscus splendidus*, Cyprinidae, Teleostei

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Study on the egg envelope of an endemic Korean freshwater fish, *Coreoleuciscus splendidus* was carried out by light and scanning electron microscopes during its spawning season. The egg envelope on the entire egg is filled with plenty of long cylinder-like villi before and after fertilization. The fertilized eggs consist of two regions, the attaching and non-attaching parts to stick to the substrates. The villi covering the fertilized eggs have three kinds of villi in its length: 1) normal-sized villi of an average of 10.6  $\mu\text{m}$  on the non-attachment part; 2) longer and more slender villi over about 20  $\mu\text{m}$  on the attachment part, at least being two times longer than those of the non-attachment; and 3) shorter villi under about 5.0  $\mu\text{m}$  around the micropyle, with half the size of the non-attachment villi. The micropyle rotated in a counter-clockwise direction with five pieces of the ridges.

**Key Words:** *Coreoleuciscus splendidus*, Egg envelope, Villi, Zona radiata, Micropyle

## INTRODUCTION

Architectures in the egg surface of various teleosts play an important role in evaluating their eggs properties as follows: the attachment of the eggs to the substratum in stream bed, and sticks or debris; the retention of water or pressure; the protection of embryo; the chorionic respiratory system; the process of water hardening (Blaxter, 1969; Wourms & Sheldon, 1976; Erickson & Pikitch, 1993; Riehl & Patzner, 1998). In addition, it has been noted that the morphological differences of the adhesive material and the associated inner layers could be a useful aid in the identification of eggs from different taxa of fish (Johnson & Werner, 1986) and as well as assist in determining the phylogenetic relationships between species (Laale, 1980; Groot & Alderdice, 1985; Riehl & Greven, 1993; Park et al., 2001; Park & Kim, 2001, 2003; Kim & Park, 2014). The Korean dace *Coreoleuciscus splendidus*, belonging to the subfamily Gobioninae in the family Cyprinidae, is an endemic freshwater fish in the Korean Peninsula (Kim &

Park, 2002). The fish inhabits the bottom of running waters with moderately fast currents (0.4~1.3  $\text{m s}^{-1}$ ) and shallow waters about 0.3~0.5 m deep in the upper and middle regions (Kim & Park, 2002). For *C. splendidus*, however, there was no information on fine structures in detail and development of egg envelope (zona radiata=egg surface=oocyte envelope=adhesive structure) covering the oocyte in this species. Therefore, the purpose of the present study is to describe the developmental features and the fine structures of the oocyte envelopes, and the relationship between their habitats and the egg envelopes in *C. splendidus*.

## MATERIALS AND METHODS

To carry out our study, 10 specimens (6 females and 4 males) were collected by between June 2011 and April 2013, spawning season, in the Han River and Mangyung River. The mature eggs are released from full-grown females by intra-abdominal pressure and they are fertilized with sperms

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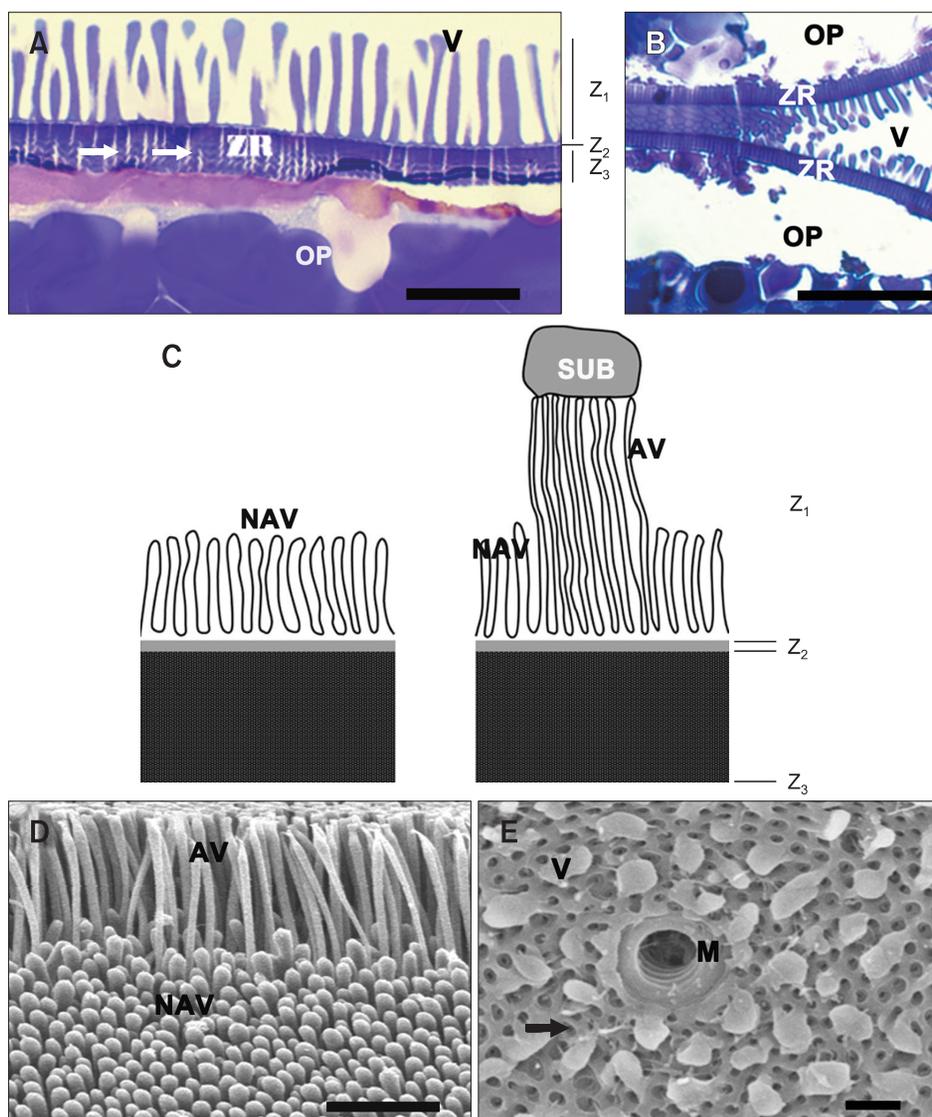
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obtained by the same method. Right after conducting artificial fertilization in beakers within about 2 minutes, the fertilized 100 eggs were fixed in 10% neutral buffered formaldehyde at pH 7.2. We dehydrated these sections through a standard ethanol series to 100%, cleared in xylene and then embedded in wax (Paraplast; Oxford, USA). Blocks were sectioned at 5  $\mu\text{m}$ . Sections were deparaffinized and stained them with Harris hematoxylin for general histology. Some sections were obtained by a semi-thin method with stock solution of Harris hematoxylin. For scanning electron microscopy (SEM), the samples were prefixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.2. Postfixation was performed in 1.0% osmium tetroxide in the same buffer. After dehydration in a graded alcohol series and drying to a critical point with liquid  $\text{CO}_2$ , the dried samples were coated with gold by ion sputtering and then examined with a Hitachi S-450 SEM

(Hitachi, Japan). For photographs and evaluation of the egg envelope, Carl Zeiss Vision was used (LE REL. 4.4; Carl Zeiss, Germany). Schematic diagrams and evaluation of the egg envelope were made by light microscopy of the hematoxylin and eosin mount preparations.

## RESULTS

In light microscopy following histologically a process of dyeing with stock solution of hematoxylin after semi-thin section, the unfertilized eggs obtained by intra-abdominal pressure from full-grown females are covered with plenty of villi on the zona radiata of the entire egg (Fig. 1). The zona radiata over ooplasm consisted of three zones, namely, outermost zona radiata ( $Z_1$ ) and middle zona radiata ( $Z_2$ ), zona radiata externa, and innermost zona radiata ( $Z_3$ ), zona radiata



**Fig. 1.** Light (A, B) and scanning electron micrographs (D, E) of the egg envelope of *Coreoleuciscus splendidus* with Harris hematoxylin staining. (A) Semi-thin section before fertilization. Scale bar=20  $\mu\text{m}$ . (B) A mass of eggs right after fertilization. Scale bar=100  $\mu\text{m}$ . (C) A schematic diagram of the villi on the egg envelope right after fertilization. (D) Villi showing an attachment part (AV) and non-attachment part on the entire fertilized egg. Scale bar=5  $\mu\text{m}$ . (E) Shorter villi around the micropyle. Scale bar=2  $\mu\text{m}$ . V, long cylinder-like villi; ZR, zona radiata; OP, ooplasm;  $Z_1$ , outermost zona radiata;  $Z_2$ , middle zona radiata (zona radiata externa);  $Z_3$ , innermost zona radiata (zona radiata interna); NAV, villi of the non-attachment villi; SUB, substrate; AV, villi of the attachment part; M, micropyle; white and black arrows, pore canals.

interna (Fig. 1A and C). In particular, the  $Z_1$  is site where villi are located, and the  $Z_3$  has different staining densities and micropores toward  $Z_2$  (Fig. 1A-C). The zona radiata was an average thickness of  $5.6 \pm 0.4 \mu\text{m}$  (range, 4.5–6.4  $\mu\text{m}$ ) ( $n=40$ ). Such long cylinder-like villi in the  $Z_1$  were hematoxylophilic with an average length of  $10.6 \pm 0.7 \mu\text{m}$  ( $n=40$ ) and an average density of  $5 \pm 1.4$  per  $10 \mu\text{m}^2$ . The fertilized eggs right after an artificial insemination formed a mass of eggs to each other and showed the same architecture, long cylinder-like villi, as unfertilized one (Fig. 1B). The eggs begin to attach to substrates such as pebbles or stones of rapid waters and a part of its egg surface swells (Fig. 1C). An attachment part where the eggs stick to substrates occurs in only a small part of the egg and its villi become longer and more slender. They are reaching more than about two times the length of the unfertilized eggs' villi (Fig. 1C and D). Meanwhile, the length of the villi in the non-attachment part covering most of the egg not adhering to the substrates remains unchanged, unlike those of the attachment part (Fig. 1C).

The micropyle of an average diameter of  $3.8 \mu\text{m}$  ( $n=4$ ) have five pieces of the ridges in the counter-clockwise direction and the pores are in cylinder shapes. There are lots of micropores that microvilli pass through. The villi surrounding the micropyle are shortened to about half the size of the non-attachment part' villi, reaching less than  $5.0 \mu\text{m}$  (Fig. 1E).

## DISCUSSION

The endemic Korean dace *C. splendidus* is generally seen in shallow waters with mostly small stony and pebbly bottoms over a small amount of sandy ground composed of one layer. Their fertilized eggs are attached to the surface of pebbles or stones of the riffle bottom (Kim & Park, 2002).

From this study, we confirmed that the egg envelope ( $Z_1$ ) on the entire egg is adorned with plenty of long cylinder-like villi before and after fertilization. It was evident that the villi covering the fertilized eggs have three kinds of villi in its length (Fig. 1C-E): 1) normal- sized villi of an average of  $10.6 \mu\text{m}$  on the non-attachment part; 2) longer villi over about  $20 \mu\text{m}$  on the attachment part, at least being two times longer than those of the non-attachment; and 3) shorter villi under about  $5.0 \mu\text{m}$  around the micropyle, with half the size of the non-attachment's villi. These characteristics in this fish were reported for the first time in this study. In particular, the attachment part to stick to substrates is not surrounded all around the egg but restricted at only a small part. To adapt to this environment, only villi on the attachment part seem to be getting longer and thinner by extension or tension. Meanwhile, the rest of the egg, the non-attachment part, may have a possibility that they attach to each other as a mass of the eggs while sticking to substrates. Similar structure is seen in a Korean gobiid fish, *Micropercops swinhonis* that attaches to

the plant stem or the surface of pebbles (Park et al., 1998, 2001). The micropyle in this species rotated in a counter-clockwise direction with five pieces of the ridges. This feature is different from other cyprinid fishes, *Zacco platypus* (ring shape) and *Hemibarbus longirostris* (funnel shape) showing the clockwise direction (Deung et al., 2000; Kim et al., 2001). We suggest that the shorter villi surrounding the micropyle, more than half the size of the non-attachment part villi, may help sperm enter the micropyle more easily. For such structures, *C. splendidus* may be closely related with its spawning habitats.

The egg envelopes, which fasten the teleostean eggs to various substrates, are not a rare phenomenon (Blaxter, 1969; Laale, 1980; Riehl & Greven, 1990, 1993; Abraham et al., 1993; Park et al., 1998; Park & Kim, 2001, 2003; Kim et al., 2011; Kim & Park, 2014). In many teleost fishes, various egg envelopes may be produced by the following materials: the follicular epithelium in the goby, *Pomatoschistus minutus* and some *Silurus* species (Kobayakawa, 1985; Abraham et al., 1993); additional layers produced by the follicular epithelium in some *Clupea* (Gillis et al., 1990); the ovarian wall in the stickleback, *Puntungia tymensis*; a special follicular epithelium in the perch, *Perca fluviatilis* (Riehl & Greven, 1993); modification of the zona radiata in Cobitidae (Riehl & Patzner, 1998; Park & Kim, 2001, 2003). In many fish taxa functions of the egg envelopes were well documented in relation to egg's properties: 1) the attachment of the eggs to the substratum in stream bed, and sticks or debris; 2) the retention of water or pressure; 3) the protection of embryo; 4) the chorionic respiratory system; and 5) the process of water hardening (Blaxter, 1969; Wourms & Sheldon, 1976; Laale, 1980; Groot & Alderdice, 1985; Riehl & Greven, 1990; Erickson & Pikitch, 1993; Thiaw & Mattei, 1996; Riehl & Patzner, 1998). In addition, the development and the structure of the egg envelopes has been used for the systematic purposes and the identification of eggs, or the evaluation of environmental factors that determine their habitat and spawning characteristics (Laale, 1980; Groot & Alderdice, 1985; Riehl & Greven, 1993; Britz et al., 1995; Thiaw & Mattei, 1996; Park & Kim, 1997, 2001, 2003; Kim et al., 2011; Kim & Park, 2014).

## CONCLUSIONS

*C. splendidus* inhabits the stones and pebbles on the bottom of rapids with swift currents and lays its eggs on such substrates. The egg envelope, therefore, is highly likely related to a spawning habitat that it lays demersal-adhesive eggs and uses this structure to prevent the fertilized eggs from slipping down or being lost or to strongly adhere to pebbles or stones with rapid waters. It shows well an adaptive strategy between the spawning and the egg envelope related to its environmental factors for this species.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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