

Adhesive Membrane of Oocyte in Korean Cobitid Species (Pisces, Cobitidae)

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Through the histological observation of ovary in the nine species of the genus *Cobitis*, we found that specific adhesive membranes were attached to the outer zona radiata during the vitellogenesis. These adhesive membranes could be classified into four forms as follows: 1) granular form of *Cobitis lutheri*, *C. striata*, and *C. sinensis*, 2) villous form of *C. longicarpus*, *C. koreensis koreensis*, *C. k. pumilus*, and *C. granoei*, 3) saw-like form of *C. rotundicaudata*, and 4) filamentous form of *C. choii*. The various forms of adhesive membrane of egg in the cobitid fishes are showed a species specificity with reference to their habitats and spawning property.

KEY WORDS: *Cobitis*, Structure, Adhesive Membrane, Zona Radiata, Oocyte

The loaches of the family Cobitidae are benthic small fishes inhabiting the freshwaters of the Europe, Asia and North Africa. The genus *Cobitis* of Korea is represented by 9 species or subspecies, of which 5 are endemic to Korea (Choi *et al.*, 1990). In the Nakdong River drainage of Korea, *Cobitis sinensis-longicarpus* hybrid complex originated from *C. sinensis* and *C. longicarpus* was reported in reference to the morphological features, karyotype with diploid and triploid and the histological features of the gonads (Kim and Lee, 1990; Kim and Park, 1993).

Although the adhesive membrane and zona radiata of some fish species were reported in relation to their substrate spawning conditions (Blaxter, 1969; Laale, 1980; Riehl and Greven, 1990, 1993), those of the cobitid fish have not been informed yet. In the course of the histological observation on the oocyte of the loaches, we found that the adhesive membranes in the outer zona radiata during vitellogenesis varied in their shape, number and size in species of the genus *Cobitis* in Korea. In this paper we plan to describe the adhesive membrane of the eggs in

fish species of the genus *Cobitis* of Korea and discuss differences with relation to their spawning habitats.

Materials and Methods

Specimens investigated in present study were used the following fishes collected from several streams of the South Korea in spawning season from 1989 to 1994: *Cobitis lutheri* Rendahl, *C. sinensis* Sauvage et Dabryi, *C. striata* Ikeda, *C. longicarpus* Kim, Choi et Nalbant, *C. granoei* Rendahl, *C. koreensis koreensis* Kim, *C. koreensis pumilus* Kim et Lee, *C. choii* Kim and Son, and *C. rotundicaudata* Wakiya et Mori.

The ovaries fixed with 10% formalin were extracted and embedded in paraffin, sectioned 5 μm and 7 μm , and stained with hematoxylin-eosin for histological experiments. Developmental stage of oocyte followed that of Nagahama (1983). The adhesive membrane investigated using the light microscope at the late yolk granules, which yolk granules are dispersed entire into cytoplasm of oocyte gradually. The number and size of adhesive

membrane also measured using micrometer within 10 μm . The term zona radiata, membrane exhibiting radial striations, was adopted terminology from Riehl and Greven (1993).

Results

Developmental process of zona radiata and appearance of adhesive membranes

Oogenesis of the genus *Cobitis* commonly occurs by proliferation of oogonia on the ovigerous lamella (Fig. 1B). After these proliferation, oogonia becomes early oocyte which consist of chromatin-nucleolus and perinucleolus (Fig. 1A, B). In perinucleolus stage, follicle cells are present but do not completely circumscribe individual oocytes, and the zona radiata was not distinct (Fig. 1B). At the stage of yolk vesicle which first appear in the important constituents of yolk materials, oocytes are surrounded by a single layer of flattened follicle cells, and the zona radiata surrounding oocyte becomes more distinct (Fig. 1C). As vitellogenesis proceeds, most of cytoplasm of oocyte become occupied by many dense yolk granules (late yolk granule) fused with each other to form yolk mass (Fig. 1D). In this period, follicle cells and zona radiata could be distinguished from outer structure of oocyte.

The adhesive membranes firstly appear in yolk vesicles but were not distinctive. But these adhesive membrane distinctively formed between zona radiata and subfollicular space in late yolk granules. These adhesive membrane and zona radiata increased in size as oocyte growths (Fig. 1D).

Adhesive membranes

The adhesive membrane of the genus *Cobitis* observed in present study could be classified into four forms: granular, villous, filamentous, and saw-like (Table 1; Fig. 2). The adhesive membrane form of the each species and their characteristics are as follows.

Granular form

The adhesive membrane of this granular form existed between zona radiata and subfollicular

space at the late yolk granules of vitellogenesis and generally showed granular features. The granules of this form were also stained with eosin (Fig. 2A). This form was included three species of *Cobitis lutheri*, *C. sinensis* and *C. striata*, however the size or number of the granule of this form was varied in some degree by the species (Table 1).

In *C. lutheri* (Table 1), these granules which was densely distributed the outer zona radiata were about 1.0 μm in diameter and 7-8 in number. The zona radiata of this species was 2.0-3.0 μm thick. But these granules of *C. sinensis* were 1.3-3.0 μm in diameter, about 2-3 in number, and randomly distributed the outer zona radiata. The thickness of zona radiata in the late yolk granules was about 2.5-3.0 μm (Table 1; Fig. 2A). *C. striata* was also showed granular form in the adhesive membrane, however the number and diameter of granules exhibit intermediate features between *C. sinensis* and *C. lutheri*. And the granules were 5-6 in number, 1.5-2.0 μm in diameter, and the zona radiata was 3.0-4.0 μm thick in this species (Table 1).

Villous form

In the four cobitid species of *C. longicorpus*, *C. granoei*, *C. koreensis koreensis*, and *C. koreensis pumilus*, unlike the granular form, the adhesive membrane was showed villus-shaped feature (Fig. 2B). These villi forms of them were also varied in the size and number each other (Table 1).

In *C. longicorpus*, the villus measuring 1.0-1.5 μm in length was located on the outer zona radiata which measures 5.0-7.0 μm at the period of late yolk granules, and the number of villi were 10-15 and densely distributed (Table 1). *C. granoei* showed short villus-shaped form that villus measured 1.5-2.0 μm in length and was 9-11 in number (Table 1). The thickness of zona radiata in this species was about 5.5-6.0 μm (Fig. 2B). The villus of *C. koreensis koreensis* measured 1.0-1.5 μm in length and was 3-4 in number. And the zona radiata was about 2.0-2.5 μm thick (Table 1). *C. koreensis pumilus* exhibited villus-shaped form with similar to that of *C. koreensis koreensis* in the number and the length (Table 1).

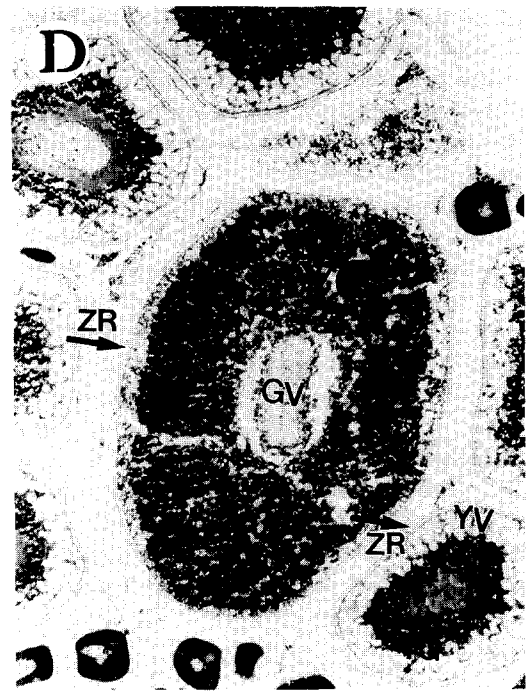
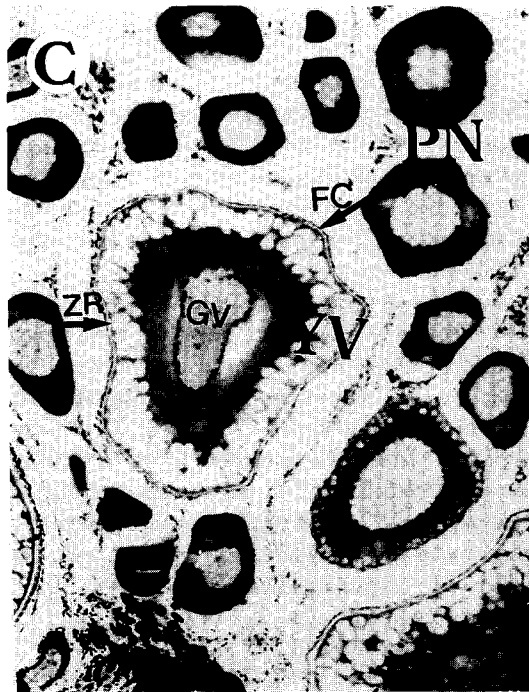
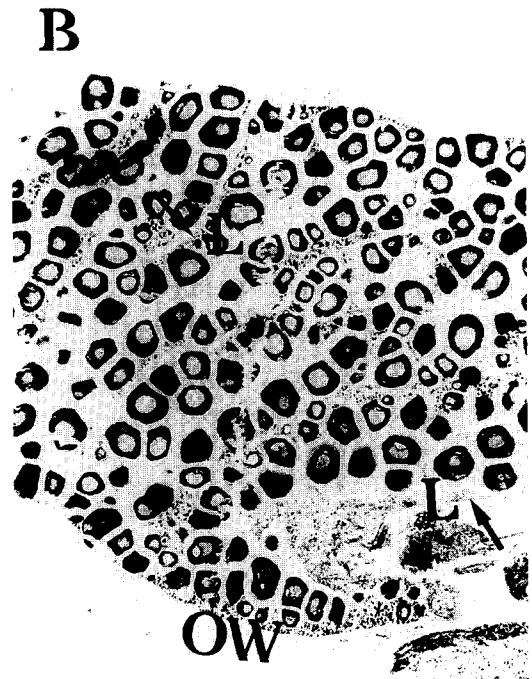


Table 1. Morphometric data of adhesive membrane of oocytes in the species of genus *Cobitis* from South Korea, based on light microscopy.

Species	Adhesive membrane			Zona radiata thickness (μm)
	Forms	Length (μm)	Number/10 μm	
<i>C. lutheri</i>	granular form	1.0	7-8	2.0-3.0
<i>C. sinensis</i>	granular form	1.3-3.0	2-3	2.5-3.0
<i>C. striata</i>	granular form	1.5-2.0	5-6	3.0-4.0
<i>C. longicarpus</i>	villous form	1.0-1.5	10-15	5.0-7.0
<i>C. granoei</i>	villous form	1.5-2.0	9-11	5.5-6.0
<i>C. koreensis koreensis</i>	villous form	1.0-1.5	3-4	2.0-2.5
<i>C. k. pumilus</i>	villous form	1.0-1.5	3-4	2.5-3.0
<i>C. choii</i>	filamentous form	3.0-4.5	4-5	5.0-6.0
<i>C. rotundicaudata</i>	saw-like form	under 0.4	12-15	3.5-4.0

Filamentous form

The adhesive membrane of *C. choii* had slender filamentous form with 4-5 in number, and 3.0-4.5 μm in length. And the thickness of zona radiata was about 5.0-6.0 μm (Table, 1; Fig. 2C).

Saw-like form

The adhesive membrane of *C. rotundicaudata* showed tiny saw-like form in the outer zona radiata. The adhesive membrane of this species was under 0.4 μm in length, and had 12-15 in number. The zona radiata of oocyte was about 3.5-4.0 μm thick (Table 1; Fig. 2D).

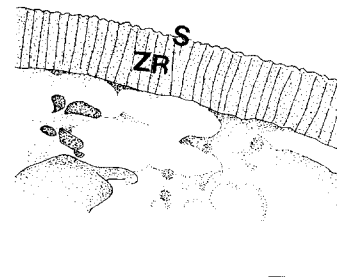
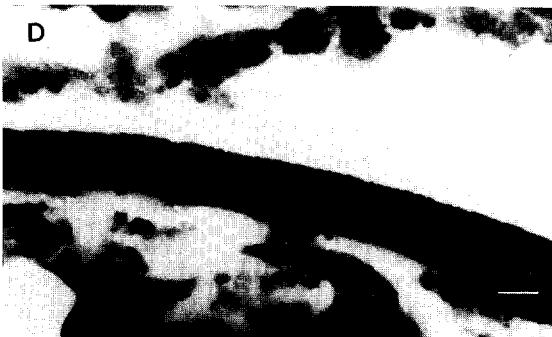
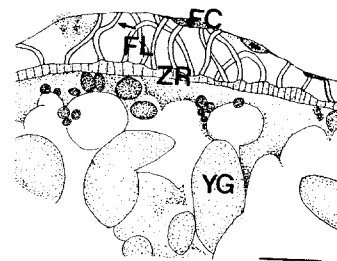
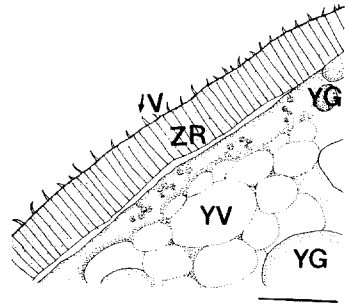
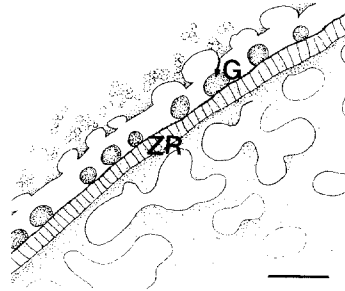
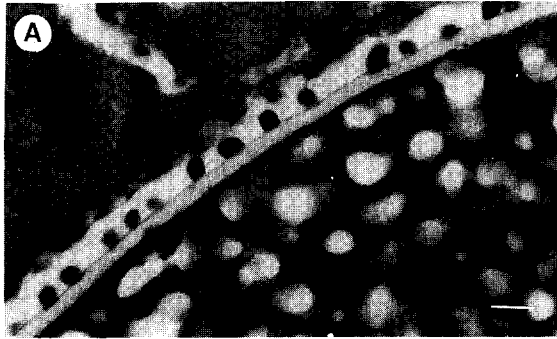
Discussion

The development and structure of oocyte in other fishes have been examined (Honma and Tamura, 1962; Gilkey, 1981; Nagahama, 1983; Hakima, 1984; Ntiba and Jaccarini, 1990; N'da and Denial, 1993), however those of the cobitid fishes still not reported. In the present study taxa, oogenesis of *Cobitis* could be largely divided into four distinct stages such as early stage with

chromatin-nucleolus and perinucleolus, vitellogenesis stage with early and late yolk vesicle, true vitellogenesis stage with early and late yolk granule, and mature stage. Follicle cells of oocyte firstly appear in early stage and become more distinct form. During vitellogenesis oocytes are surrounded by a single layer of flattened follicle cells. The zona radiata, membrane exhibiting radial striations, become visible in the period of yolk vesicle, and as vitellogenesis proceeds they become more distinct and increased in size. The results on formation of the zona radiata and growth of oocyte in the genus *Cobitis* well accorded with results reported by other authors (Worums, 1976; Wallace and Selman, 1981; Nagahama, 1983; Groot and Alderdice, 1984; Kjorsvik and Davenport, 1984). The term zona radiata has been used various synonyms such as chorion (Kjesbu and Kryvi, 1989), egg membrane (Nagahama, 1983), egg envelope (Riehl and Greven, 1993), vitelline membrane and vitelline envelope (Riehl and Greven, 1993; Selman and Wallace, 1986).

The adhesive membranes are attached to zona radiata of full-grown oocytes (Laale, 1980; Kjesbu and Kryvi, 1989; Riehl and Greven, 1993).

Fig. 1. Developmental stage of ova in *C. sinensis* from Korea. A. Oogonia (O), which undergo proliferation by mitotic divisions and chromatin-nucleolus stage (CN), which is surrounded by conspicuous nucleolus. PN; perinucleolus. $\times 1,000$. B. Early oocytes of perinucleolus stage (PN) proliferate on the ovigerous lamellae (L) of ovary surrounded ovarian wall (OW). $\times 80$. C. Flattened follicles cells (FC) and zona radiata (ZR) become visible in the yolk vesicle (YV) stage which is first yolk materials. GV; germinal vesicle, PN; perinucleolus. $\times 100$. D. Most of cytoplasm of oocyte become occupied by many dense yolk granules (late yolk granule, YV) fused with each other to form yolk mass (Fig. 1, D). In this period, the zona radiata (ZR) become more distinctive. GV; germinal vesicle, YV; yolk vesicle stage. $\times 80$.



Honma and Tamura (1962) observed that *Plecoglossus altivelis* displayed a large number of fence-shaped adhesive membranes on the outer zona radiata of animal poles. *Oryzias latipes* and some species of Cyprinodontiformes displayed filament-shaped adhesive membrane (Riehl and Greven, 1993; Hart *et al.*, 1984). Some species of *silurus* and *Pungitius tymensis* are known adhesive membrane which are surrounded by a gellatinous mass (Kobayakawa, 1985). In addition, various patterns of adhesive membrane immediately before or after fertilization were reported by Laale (1980): hexagonal pattern in *Cynolebias ladogesi*, bead-like granules in *Clinocottus recalvus*, button-shaped appendage in *Apeltes quadracus*, Wart-like appendage in some of *Pleuronichthidae*, and adhesive disc consisting of a bundle of threads or filaments in some *gobius*.

The benthic loaches inhabited different habitats, such as pebble or sand, or mud bottom by the species (Kim and Son, 1984; Kim and Lee, 1988; Kim and Kang, 1993). Through the histological observation of oocytes in almost species of *Cobitis*, we founded that various forms of adhesive membrane are attached to the outer zona radiata during vitellogenesis. In the genus *Cobitis*, these adhesive membranes could be divided into four forms: 1) granular form, 2) villous form, 3) filamentous form, and 4) saw-like form. Such a various adhesive membranes are also deeply related to their habitats. Granular form found in species, such as *C. sinensis*, *C. lutheri*, and *C. striata* which inhabit almost sandy bottoms, whereas *C. longicorpus*, *C. granoei*, *C. koreensis koreensis*, and *C. k. pumilus* having villous form inhabited pebbly bottoms.

Filamentous form found in *C. choii* which is buried deep in sand. And saw-like form found in *C. rotundicaudata* which inhabit pebbly bottoms of the upper streams connecting rapids and marsh.

Even if the form of adhesive membrane seemed to be similar with each other in cobitid species, it was clearly recognized that the size or the number of adhesive membrane varied by species. In the granular form, we found a difference that in number of the granules *C. lutheri* was 7-8, *C. striata* was 5-6, and *C. sinensis* was 2-3, and that in the size of granules *C. sinensis* was 1.3-3.3 μm , *C. striata* was 1.5-2.0 μm , and *C. lutheri* was 1.0 μm . In the villous form, we could also observe differences in numbers or length of villi by species. *C. choii* inhabited deep on sands has filamentous adhesive membrane in oocyte differing from the granular form of some cobitid species which lived on sands. This filamentous structure of oocytes in *C. choii* presumably reduces the probability of being buried by slight shifts in the sand and keeps the embryos in well-oxygenated water. For this morphological differences, we consider that *C. choii* may be differ from other cobitid species in the spawning habitat. *C. rotundicaudata* exhibited tiny adhesive membrane as saw-like form. Such a saw-like form seems also to be in refer to their benthic spawning habitats.

Filament-like adhesive membrane have been known from egg of substrate-spawning teleosts (Blaxter, 1969; Kjesbu and Kryvi, 1989; Riehl and Greven, 1990, 1993). Also Laale (1980) suggested that the adhesive membrane consisted of several materials as mucus, mucine, and mucilage, or gelatin, and that various adhesive membranes have adhesive properties enables the

Fig. 2. Light micrographs (left) of various adhesive membrane on the outer zona radiata of the genus *Cobitis* from South Korea and schematic diagrams (right) that magnify middle part of light micrograph (Left). A. Granular form, which existed between zona radiata and subfollicular space at the late yolk granules. *C. sinensis*: granules of this form are 2-3 in number/10 μm and 1.3-3.0 μm in diameter. G; granular form, ZR; zona radiata. Bar is 10 μm . B. Villous form, which is attached to the outer zona radiata. *C. granoei*: villi are 9-11 in number/10 μm and 1.5-2.0 μm in length. V; villous form, YG; fused yolk granules, YV; yolk vesicles, ZR; zona radiata. Bar is 10 μm . C. Filamentous form. *C. choii* had slender filament-like form that the length are far longer than those of villous form. These filaments are 4-5 in number/10 μm and 3.0-4.5 μm in length. FC; follicle cell, FL; filamental form, YG; fused yolk granules, ZR; zona radiata. Bar is 10 μm . D. Saw-like form. This structure of *C. rotundicaudata* seems likely to be rather a modifications of the outer zona radiata than a adhesive membrane attaching to the outer zona radiata like other fishes of *Cobitis*. The saw were under 0.4 μm in length and 12-15 in number/10 μm . S; Saw-like form, ZR; zona radiata. Bar is 10 μm .

eggs to become attached to vegetation, submerged objects, and to one another. In the American smelt (*Osmerus mordax*), the egg has a low stalk which is adhesive and becomes attached to the stony bottoms of streams where the species most often spawns (Lagler *et al.*, 1977). In the brook silverside (*Labidesthes sicculus*), the egg has a single elongate filament that serves first for temporary flotation and then for attachment (Lagler *et al.*, 1977). Also fishes having adhesive membrane are most nonbuoyant eggs (Blaxter, 1969; Lagler *et al.*, 1977). Morphological aspects of these zona radiata have occasionally been used for taxonomic purposes (Riehl, 1980; Jonson and Werner, 1986). In filamental structures, a difference in size, distribution, and number was useful and have been used for distinction of property of egg (Laale, 1980).

Such a various adhesive membranes in the cobitid fishes must be related to the spawning substrates and properties of egg, and these differences also exhibited species specificity. So we believe that the adhesive membrane of oocyte in the cobitid fishes could be used as a taxonomic character for the species identification. In future, fine structure on the zona radiata and the adhesive membrane of fishes which comes to question may be a great help to understand biological features of the fish.

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한국산 기름종개속 어류의 난모세포의 부착막
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한국의 기름종개속 *Cobitis* 어류 9종의 난모세포 부착막(adhesive membrane)을 조직학적으로 조사한 결과, 난황물질 형성시기인 난황구 후기의 zona radiata 외측에는 다음 4가지 형태의 부착막이 구별되었다. *Cobitis lutheri*, *C. striata*, *C. sinensis*는 과립형(granular form)의 부착막을 보여 주었으며 *C. longicarpus*, *C. koreensis*, *C. k. pumilus*, *C. granoei* 등의 4종은 융모형(villous form)을 나타냈다. 그리고 *C. rotundicaudata*는 거치형(saw-like form)을 보여 주었으며 *C. choii*는 사상형(filamentous form)을 나타냈다. 이상과 같이 기름종개속 어류의 알에서 나타나는 다양한 형태의 부착막들은 그들의 서식처와 산란성질에 대해서 종의 특이성을 보여 주었다.