Korean J. Ichthyol. 13(1), 19~23, 2001

Ultrastructure of the External Egg Envelopes in Two Cobitid Fishes (Cobitidae)

Jong-Young Park and Ik-Soo Kim

Faculty of Biological Sciences, College of Natural Sciences, Chonbuk National University, Chonju 561-756, Korea

The full-grown oocyte envelope of the two spined loaches, *Niwaella multifasciata* and *Kichulchoia brevifasciata*, was examined by electron microscopy. The oocyte is surrounded by its envelope, and an external modification (an adhesive structure) in the surface of the envelope is present. The envelope consists of two layers, a zona radiata externa which is the site of the adhesive structure, and a zona radiata interna, which has heterogeneous, electron-dense multi layers. The surface (zona radiata externa) of the envelope in *N. multifasciata* is equipped with short villus-like protuberances, which have a length of 1.5~2.5 μm and are separated from each other by a distance of 2~2.5 μm. In contrast, *K. brevifasciata* has undulating or wave-like structures that extend over the entire oocyte surface. The waves are 1.5~2.5 μm in length and are separated a distance of 2.5~3.3 μm from each other.

Key words : *Niwaella, Kiculchoia*, egg envelope, zona radiata, villus, wave

Introduction

A number of teleost fishes deposit demersal eggs on a substrate. Among them, many fish eggs have adhesive properties which enable the eggs to become attached to the substrate and to one another (Yorke and McMillan, 1979; Laale, 1980; Abraham *et al*., 1993; Thiaw and Mattei, 1996; Riehl and Patzner, 1998). Attachment mechanism of eggs varies according to species and is associated with particular substrates as well as with spawning conditions (Blaxter, 1969; Laale, 1980; Riehl and Greven, 1990, 1993). The external appearance of the envelope may be a useful character in the determination of systematic relationships (Laale, 1980; Groot and Alderdice, 1985; Riehl and Greven, 1993; Britz *et al*., 1995; Park and Kim, 1997, 2001; Thiaw and Mattei, 1996).

Two cobitid fishes, *Niwaella multifasciata* and *Kichulchoia brevifasciata*, are small and benthic freshwater fishes that deposit their eggs on substrates such as pebble stone. The genus of *Kichulchoia brevifasciata* was transformed from *Niwaella* to *Kichulchoia* by Kim *et al*. (1999) based on their morphological characters. Therefore, we describe the structure of egg envelopes and also discuss the relationship between the habitat and the envelope of two cobitid fishes, *N. multifasciata* and *K. brevifasciata*.

Materials and Methods

Females of two cobitid fishes, *Niwaella multifasciata* and *Kichulchoia brevifasciata* were collected from several streams in South Korea during the spawning season, December (*N. multifasciata*) and June (*K. brevifasciata*).

For transmission electron microscopy (TEM) and scanning electron microscopy (SEM), adult gravid females were anaesthetized with MS222. Their ovaries were excised and prefixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2. Postfixation was performed in 1% osmium tetroxide in the same buffer. After dehydration in a graded alcohol series, specimens were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed with JEOL-1200EX transmission electron microscope.

For scanning electron microscopy (SEM), ovaries were prefixed and postfixed in same way as those for TEM. The samples were dehydrated in a graded alcohol series and critical point dried in CO_2. The dried samples were coated with gold-palladium. Normal eggs and eggs with the follicle-removed were observed with a JEOL JSM-T330A scanning electron microscope.

Results

In *Niwaella multifasciata* and *Kichulchoia brevifasciata*, the full-grown oocyte consists of

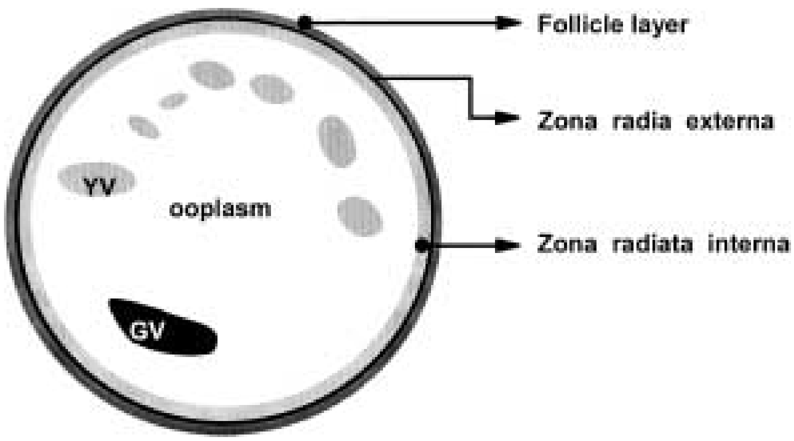

Fig. 1. A schematic diagram of light microscopy from the full-grown oocyte of *Niwaella* and *Kichulchoia* (not in proportion). GV, germinal vesicle; YV, yolk vesicle.

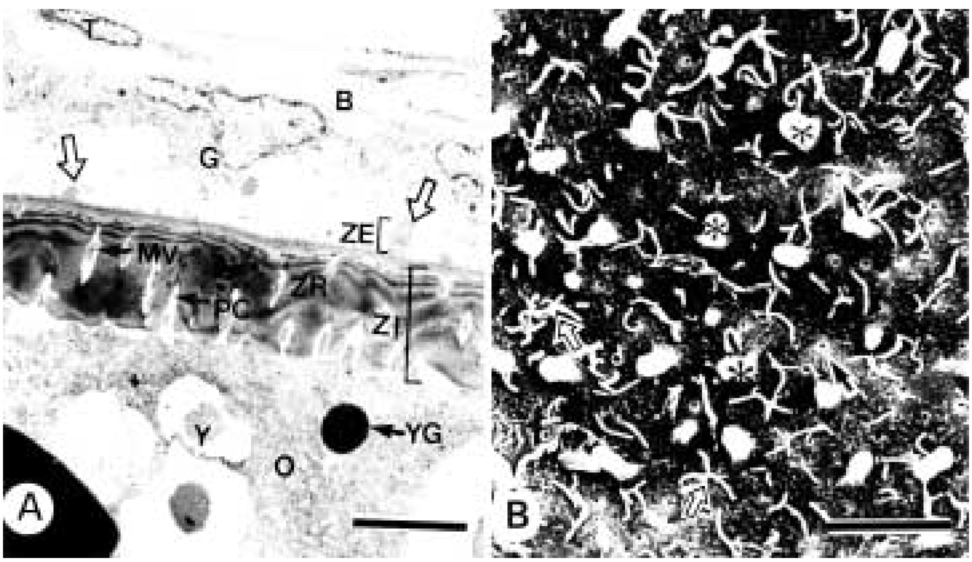

Fig. 2. Electron micrographs of the oocyte of *Niwaella multifasciata*. A: Transmission electron micrograph of a cross section. The zona radiata (ZR) consists of a zona radiata externa (ZE) and a zona radiata interna (ZI). The zona radiata externa is site of short, adhesive short villi (White arrows) which show a lesser electron-density. The zona interna consists of five to seven layers which have an heterogeneous electron-density. The microvilli (MV) project through the pore canals (PC) throughout zona radiata toward the granulosa cell (GC). The ooplasm (O) consists of yolk materials, yolk vesicles (Y) and yolk globules (YG) with a marked electron-density. The outermost layer, the follicle layer consists of theca cells (T) and granulosa cells (G) separated by basement membrane (B). Scale bar = 10 μm. B: Scanning electron micrograph of an follicle-removed oocyte. The short villus-like protuberances (*) surround the oocyte and microvilli (white arrows) extend through the pore canals (black arrows) are founded. Scale bar = 5 μm.

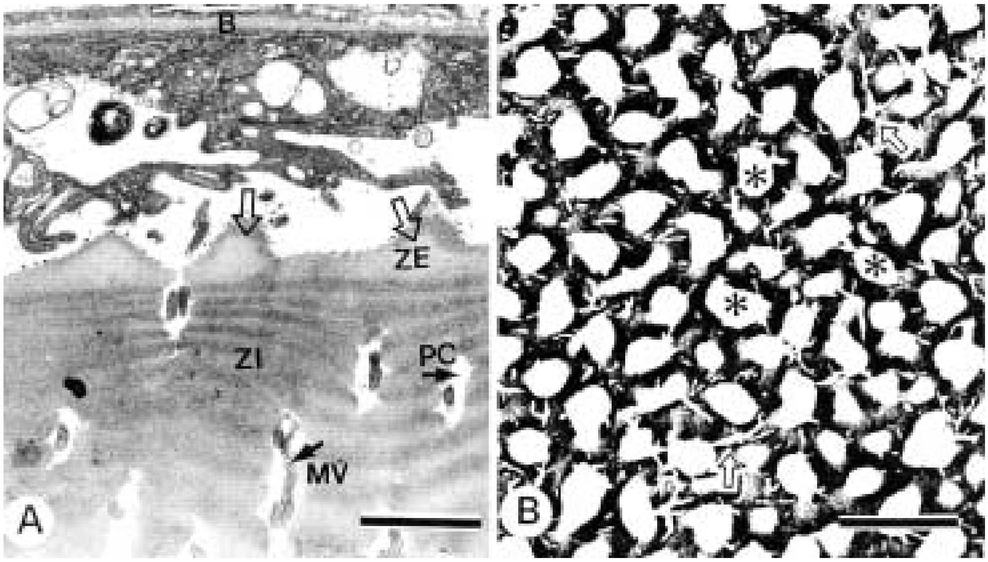

Fig. 3. Electron micrographs of the oocyte of *Kichulchoia brevifasciata*. A: Transmission electron micrograph of a cross section. An undulating or a wave−like structure (white arrow) is located at the zona radiata externa (ZE). The microvilli (MV) extending through pore canals (PC) project from the zona radiata interna (ZI) toward the follicle layer. The zona radiata interna displays twelve heterogeneous electron−dense layers. The microvilli project from the oocyte toward the granulosa cell through the pore canals that traverse the zona radiata. B, basement membrane. Scale bar = 1 μm. B: Scanning electron micrograph of an follicle−removed oocyte. The oocyte surface is covered with undulating ridges (*). Microvilli occur among the villi. Scale bar = 5 μm.

an outermost layer (follicle layer), envelope (zona radiata) and the yolk materials, i.e. yolk vesicle and yolk granule of the oocyte proper (Figs. 1 to 3). The follicle layer consists of theca cells and granulosa cells which are separated by the basement membrane (Figs. 2A, 3A).

Surface structures of the zona radiata

The surface structures of the zona radiata show a clear difference in their appearance: short villus−like protuberances in *N. multifasciata* and undulating or wave−like structures in *K. brevifasciata*.

Niwaella multifasciata

The villi have a length of 1.5～2.5 μm and are spaced at a distance of 2.0～2.5 μm from each other. In transmission electron micrograph (Fig. 2A), the villi, which are of a lesser electron−density, are located at zona radiata externa and extend to the granulosa cells. The follicle layer is divided into an outer theca cell layer and inner granulosa cell layer by a basement membrane. The zona radiata interna is 5.0～7.0 μm in thickness and usually consists of five to seven layers that exhibit an heterogeneous electron−density. Microvilli project through the pore canals of the zona radiata toward the granulosa cells. In scanning electron micrograph (Fig. 2B), the villus−like protuberances are spread over the entire egg surface, and many microvilli occur among the villi.

Kichulchoia brevifasciata

The protuberances are approximately 1.5～2.5 μm in length and occur at a distance of 2.5～3.3 μm from each other. In transmission electron micrograph (Fig. 3A), the zona radiata externa, which is the site for the adhesive structure, exhibits a more or less electron−dense layer. The zona radiata interna is composed of twelve heterogeneous electron−dense regions whose individual thickness is approximately 3.5～4.5 μm. Microvilli pass through the pore canals from the zona radiata to the granulosa cells. In scanning electron micrograph (Fig. 3B), the egg surface is covered with numerous small adhesive ridges and many microvilli.

Discussion

The envelopes of two cobitid species, *Niwaella multifasciata* and *Kichulchoia brevifasciata*, show clear differences in appearance: the former has short adhesive villi, and the latter has undulating or wave-like ridges. The difference in the surfaces of the two envelopes may be associated with differences in particular spawning substrates as well as with spawning conditions. *N. multifasciata* inhabits regions large, with a pebble bottom in rapidly flowing bodies of water, whereas *K. brevifasciata* inhabits regions small, with a pebble bottom in slow currents or stagnant water of shallow depth. In response to these ecological differences, they seem to have special adhesive structures, which enable the ovulated eggs to attach to the substrates. A relationship between the habitat and the structure of envelopes has been reported in some cobitid fishes (Kim and Park, 1995, 1996; Park and Kim, 1997, Park and Kim, 2001). Although several species had envelopes that were superficially similar in appearance, they exhibited differences in the length, number, or ultrastructure of ornamentation.

The main function of the structures associated with the envelope that surrounds the egg is for the attachment of the eggs to the substratum (Blaxter, 1969; Laale, 1980; Riehl and Greven, 1990, 1993; Park *et al*., 1998; Riehl and Patzner, 1998). In addition to the attachment function of the envelope, various other functions are known. (1) The polygonal ornaments in *Epiplatys* may retain water and may alter the egg buoyancy (Thiaw and Mattei 1996). (2) The mucous coat of the sheatfish *Silurus glanis* L swells, becomes buoyant and adhesive, thus facilitating adherence of the eggs to the nest wall and to each other (Abraham *et al*., 1993). (3) It serves as a chorionic respiratory system in *Cynolebias* (Wourms and Sheldon 1976). (4) The attaching filaments offer the advantages of a better supply of oxygen in pikes *Esox lucius* and *E. masquinony*, an improvement of the chances of fertilization and a protection of the clutch (Riehl and Patzner, 1998). Except for the attachment function, other functions have not been demonstrated in *N. multifasciata* and *K. brevifasciata*.

In our study, the thickness of the zona radiata is about 5.0 to 7.0 μm in *N. multifasciata* and 3.5 to 4.5 μm in *K. brevifasciata*. In *Oryzias latipes*, the zona radiata is 12～15 μm thick (Hart *et al*., 1984), in *Cynolebias melanotaenia*, 4.5 μm thick (Wourms, 1976), and in *Oncorhynchus* and *Salmo*, 28～62 μm and 31～50 μm, respectively (Groot and Alderdice, 1985; Riehl, 1991). In viviparous Goodeidae and Poeciliidae, the zona radiata is considerably thinner (0.3 to 2.0 μm) (Riehl and Greven, 1993). It has been reported that the reduction in thickness of the zona radiata is a response to the need for gaseous exchange between the embryonic and maternal tissues in viviparous vertebrates (Riehl and Greven, 1993). Differences in the structure of the primary membrane (zona radiata) is indicative of adaptations to spawning and egg development, and that structure of the zona radiata is closely related to environmental factors and systematic relationships (Ivankov and Kurdyayeva, 1973; Hirai, 1993).

References

Abraham, M., V. Hilge, R, Riehl and Y. Iger. 1993. Muco-follicle cells of the jelly coat in the oocyte envelope of the sheatfish (*Silurus glanis* L.). J. Morphol., 217 : 34～43.

Blaxter, J.H.S. 1969. Development: eggs and larvae. In: Hoar, W.S., D.J. Randall and E.M. Donalson, (eds), Fish physiology III. Academic Press, New York, pp. 177～252.

Britz, R., M. Kokoscha and R. Riehl. 1995. The anabantoid genera *Ctenops*, *Luciocephalus*, *Parasphaerichthys*, and *Sphaerichthys* (Teleostei: Perciformes) as a monophyletic group: evidence from egg surface structure and reproductive behavior. Japan. J. Ichthyol., 42 : 71～79.

Groot, E.P. and D.F. Alderdice. 1985. Fine structure of the external egg membranes of five species of Pacific salmon and steelhead trout. Can. J. Zool., 63 : 552～566.

Hart, N.H., R. Abraham and M. Donovan. 1984. The structure of the chorion and associated surface filaments in *Oryzias*; evidence for the presence of extracellular tubules. J. Exp. Zool., 230 : 273～296.

Hirai, A. 1993. Fine structure of the egg membrane in four species of Pleuronectinae. Japan. J. Ichthyol., 40 : 227～235.

Ivankov, V.N. and V.P. Kurdyayeva. 1973. Systematic differences and the ecological importance of the membranes in fish eggs. J. Ichthyol., 13 : 864～873.

Kim, I.S. and J.Y. Park. 1995. Adhesive membranes of oocyte in Korean cobitid species (Pisces, Cobitidae). Korean J. Zool., 38 : 212～219.

Kim, I.S. and J.Y. Park. 1996. Adhesive membrane of oocyte in four loaches (Pisces: Cobitidae) of Korea. Korean J. Zool., 39 : 198～206.

Laale, H.W. 1980. The perivitelline space and egg envelopes of bony fishes; a reviews. Copeia (1980) : 210～226.

Kim, I.S., J.Y. Park and T.T. Nalbant. 1999. The far-east species of the genus *Cobitis* with the

description of three new taxa (Pisces, Cobitidae, cobitidae). Trav. Mus. Natl. Hist. Nat (Grigora Antipa)., 39 : 373～391.
Park, J.Y. and I.S. Kim. 1997. Egg membrane in five cobitid species of *Cobitis* (Pisces: Cobitidae). Korean J. Ichthyol., 9 : 121～129.
Park, J.Y., K.C. Richardson and I.S. Kim. 1998. Developmental changes of the oocyte and its enveloped layers in *Micropercops swinhonis* (Pisces, Perciformes). Korean J. Biol. Sci., 2 : 501～506.
Park, J.Y. and I.S. Kim. 2001. Fine structures of oocyte envelopes of the related three cobitid species in the genus, *Iksookimia* (Cobitidae). Ichthyol. Res., 48 : 71～75.
Riehl, R. and H. Greven. 1990. Electron microscopical studies on oogenesis and development of egg envelopes in two viviparous teleosts, *Heterandria formosa* (Poeciliidae) and *Ameca splenens* (Goodeidae). Zool. Beitr., 33 : 247～252.
Riehl, R. 1991. Structure of oocytes and egg envelopes in oviparous teleost-an overview. Acta. Biol. Benrodis, 3 : 27～65. (In German)
Riehl, R. and H. Greven. 1993. Fine structure of egg envelopes in some viviparous goodeid fishes, with comments on the relation of envelope thinness to viviparity. Can. J. Zool., 71 : 91～97.
Riehl, R. and R. Patzner. 1998. Minireview: the modes of egg attachment in teleost fishes. Ital. J. Zool., 65 : 415 ～420.
Thiaw, O.T. and X. Mattei. 1996. Ultrastructure of the secondary egg envelope of Cyprinodontidae of the genus *Epiplatys* Gill, 1862 (Pisces, Teleostei). Acta Zool., 77 : 161～166.
Wourms, J.P. 1976. Annual fish oogenesis. I. Differentiation of the mature oocyte and formation of the secondary egg envelope. Devel. Biol., 50 : 355～366.
Wourms, J.P. and H. Sheldon. 1976. Annual fish oogenesis. II. Formation of the secondary envelopes. Devel. Biol., 50 : 338～354.
Yorke, M.A. and D.B. McMillan. 1979. Nature and cellular origin of the adhesive coats of the lamprey egg (*Petromyzon marinus*). J. Morphol., 162 : 313～326.

Received : January 10, 2001
Accepted : March 4, 2001

미꾸리과 어류 2종에 대한 난막의 미세구조

박 종 영 · 김 익 수

(전북대학교 생물과학부)

미꾸리과 어류 2종 수수미꾸리 *Niwaella multifasciata*, 좀수수치 *Kichulchoia brevifasciata*의 성숙란에 대하여 난막구조를 조사하였다. 성숙란의 난막 (egg envelope)은 부착구조 (adhesive structure)가 부착하는 zona radiata externa와 여러 전자밀도층을 가지는 zona radiata interna층으로 구성되어 있다. 수수미꾸리의 zona radiata externa는 짧은 융모형태 (short villus-like protuberance)의 구조를 가지며 길이는 약 1.5～2.5 μm로서 2.0～2.5 μm 간격으로 배열하고 있었다. 반면에 좀수수치는 물결모양 형태 (wave-like structure)를 가지는데 길이는 약 1.5～2.5 μm 크기로 서로 2.5～3.3 μm 간격으로 배열되어 있다.