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Structure on the Ventral Process and the Ovipositor of a Korean Oily Bitterling, *Acheilognathus koreensis* (Pisces, Cyprinidae), in Relation to Spawning Season

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During spawning season of female in a Korean oily bitterling, *Acheilognathus koreensis*, the ventral region near the base of the pectoral fin becomes to be protruded outward of body and enlarged. This ventral process consists of both organs as rectum (vent) and inner ovipositor. The rectum consists of mucosa, lamina propria-submucosa, muscularis, and squamous epithelial layer (peritoneum=serosa) surrounding them. The mucosa contains numerous mucous cells meaning acid mucopolysaccharides in nature. The inner ovipositor is similar to that of the rectum, but the mucosa have no mucous cell, unlike that of the rectum. Whereas, the outer ovipositor has a straight and long tube which are not connected with the ventral process any more. The outer ovipositor was similar to the structure of the inner ovipositor in the ventral process. However, the outer ovipositor has no muscularis, and consists of three layers: mucosa, lamina propria-submucoa, and squamous epithelia. The outer ovipositor without the muscularis seems serves as a tube that eggs discharged from the outer ovipositor allow to send inside mussel, unlike that of the inner ovipositor performing rhythmic contractions of the layers of the muscularies for propelling to the matured oocytes to the outer ovipositor.

Key words : Bittering, *Acheilognathus*, ventral process, ovipositor, rectum

Introduction

The bittering, subfamily Acheilognathinae, is a small freshwater fish with deep body and a semi-inferior mouth. They are distributed in temperate regions of Europe and Asia, including Korea, Japan, Taiwan, and China (Nelson, 1984; Berra, 2001; Kim and Park, 2002; Kim *et al*., 2005). During the breeding season, it was widely known that the female possesses a long ovipositor behind its anal opening that they use to place their eggs onto the gills of a mussel. The male fertilizes the eggs by releasing sperm into the inhalant siphon of the mussel, and the fertilized eggs develop inside the mussel gill cavity and leave the mussel as actively swimming larvae. These co-evolution between a European bitterling *Rhodeus* and freshwater mussels was reported (Heschl, 1989; Reynolds and Guillaume, 1998; Aldridge, 1999; Candolin and Reynolds, 2001; Mills and Reynolds, 2003; Smith *et al*., 2004). And there were many reports on their taxonomy, ecology, and development of free larvae (Nakamura, 1969; Arai and Akai, 1988; Suzuki and Jeon, 1991; Kim and Park, 2002; Kawamura and Uehara, 2005; Kim *et al*., 2005). The Korean bittering had 3 genera and 14 species including 9 endemic species (Kim *et al*., 2005), and many researches were performed on several fields such as osteology, sexual maturation and spawning, development, feeding habits, and systematics

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(Kim and Kim, 1990, 1991; Suzuki and Jeon, 1991; Song and Kwon, 1994; Kim and Yang, 1998; Arai *et al*., 2001; Baek *et al*., 2002, 2003; Baek and Song, 2005; Song and Son, 2005).

Until now, researches on bittering were focused on morphological, physiological and behavioral aspects as listed above. However, although the ovipositor of the female is an important reproductive organ during spawning season in bittering, histological approaches on ovipositor of female were few. Therefore, through histological study on the ovipositor of Korean oily bitterling, *Acheilognathus koreensis*, known as a Korean endemic species, it is to report its basic structure including genital pore and get any information on interspecific differences of subfamily Acheliognathinae in future.

Materials and methods

The observed 3 specimens were collected during the spawning season (May to June), from the Gwancho-myon, Imsil-gun, Jeonllabuk-do, (a tributary of Somjin River), Korea, 2005 and ranged from 56.5 mm to 60.4 mm in standard length. The genital pore and ovipositor fragments for histological examination were fixed in 10% neutral buffered formaldehyde. The fragments were taken from 4 different regions: anterior ventral process with ovipositor just near the base of the anal fin, posterior ventral process with ovipositor just near the base of the anal fin, anterior ovipositor and posterior ovipositor without ventral process (Figs. 1 and 2).

These fragments were dehydrated through a standard ethanol series to 100%, cleared in xylene and then embedded in wax (Paraplast, Oxford). 5 μm sections were deparaffinized and stained with Ehrlich haematoxylin, counter-stained with eosin, and Masson trichrome stain (Gurr, 1956) for general histology. Mucin of gland were demonstrated by alcian blue solution (AB) at pH 1.0 and 2.5 (Steedman, 1950; Lev and Spicer, 1964), and periodic acid-Schiff (PAS) method (Lilllie and Greco, 1947). For evaluations of the tissue and intestine, we used Carl Zeiss vision (LE REL. 4.4). The measurement of standard length and ovipositor length used 1/20 mm digital calipers.

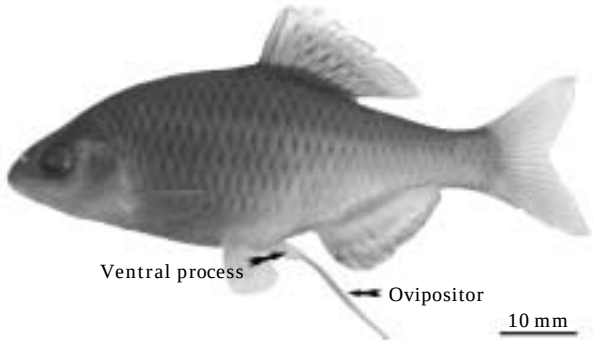

Fig. 1. An overall morphology of the ventral process and ovipositor of female during the spawning season of a Korean oily bittering, *Acheilognathus koreensis*.

Results

1. External morphology of ventral process and ovipositor

1) Ovipositor in breeding season

The ventral regions near the base of the pectoral fin has the ventral process (a protruded anus) and the ovipositor consisted of two parts such as the inner ovipositor part connected with the ventral process and the outer ovipositor with a long and slender tubule without the ventral process (Figs. 1 and 2).

Acheilognathus koreensis has an ovipositor as a tubular egg-laying organ behind ventral process which is situated between the base of the pelvic fins and just in front of the anal fin in the breeding season. As the breeding season comes, the ovipositor begins to extend and reaches to the anal fin at a peak time of the breeding season for depositing eggs in the gill cavity of the pond mussel (Figs. 1 and 2).

The ovipositor including the ventral process is about 20.5 to 23.8 mm in length and the pure ovipositor excluding the ventral process about 15.2 to 18.5 mm.

2) Ovipositor in non-breeding season

In non-breeding season, the ovipositor and ventral process is not protruded out body, as depressed into the body cavity. The ventral region has two pores, anus and urogenital pore.

The ventral region of male throughout the year has no process, and there are just two pores like a female in the non-breeding season.

2. Internal structure

1) Ventral process with inner ovipositor

This region is just near the base of the pectoral fin (Figs. 1 and 2) and has inner ovipositor behind the ventral process. The ventral process con-

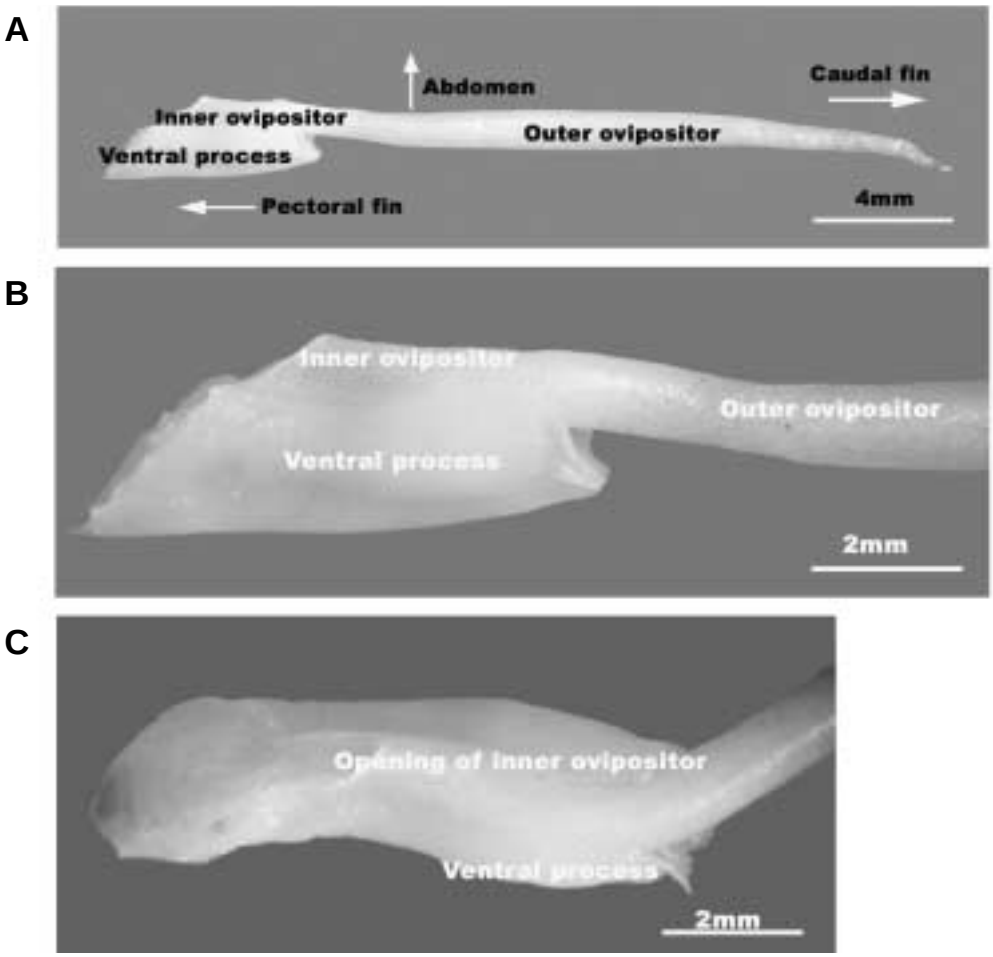

Fig. 2. An external morphology of the ventral process and ovipositor of female during the spawning season of a Korean oily bittering, *Acheilognathus koreensis*.

sisted of outer stratified squamous epithelia of an ventral region, vent (rectum) and inner ovipositor (Fig. 3A to 3C). The vent and inner ovipositor has an opening connected with rectum and the oviduct from body cavity, which is called anus and urogenital organ, respectively.

(1) Outer stratified squamous epithelium of the ventral region

The ventral region consisted externally of multi-layered squamous epidermal cells for giving a serosa, the outermost layer of the rectum and the inner ovipositor (Fig. 3A to 3E). The ventral region of the swollen part toward ventral view in external morphology was protruded as a large plug just over the rectum (Fig. 3A and 3E). The plug structure has the thickest muscle of circular or spiral form. The outer stratified epithelial layer is thicker around the base of the pectoral fin, but thinner toward caudal fin.

This epithelial layer has a basal layer and a stratified layer (Fig. 3D). The basal layer consisted of cuboidal or cylinder cell, not columnar cell, and a few lymphocytes. The stratified layer is composed of small round, ovoid, or a few flat cells. Lots of mucous cells are present in the superficial region, and sometimes in the middle layer. These mucous cells give a deep red color reaction with PAS technique, blue with the AB at pH 1.0 and 2.5 (Fig. 3D). Their nuclei are purple or red color with AB-PAS reaction and Masson trichrome stain. These mucous cells are acid mucopolysaccharides in nature. The mucous cell is small spherical or flask-shaped unicellular mucous glands were discernible (Fig. 3D). They have a large spherical body and a short narrow neck that opens on the surface by a wide pore.

(2) Vent (rectum)

The rectum is situated at the middle region of

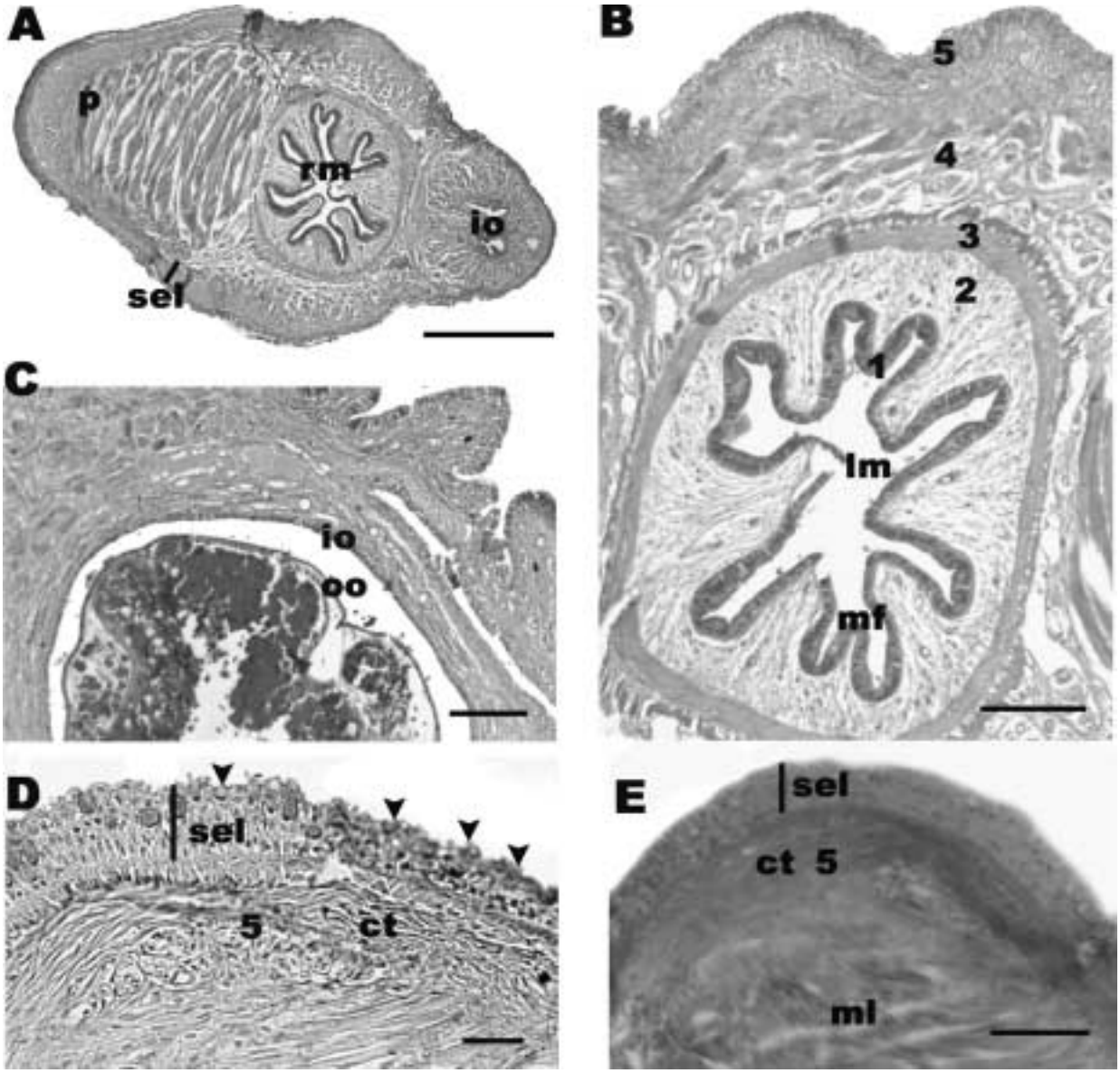

Fig. 3. Transverse section of the ventral process and ovipositor of *Acheilognathus koreensis*. A, Overall structure, Ehrlich haematoxylin and eosin (bar=0.5 mm); B, Rectum, Ehrlich haematoxylin and eosin (bar=0.2 mm); C, Inner ovipositor, Ehrlich haematoxylin and eosin (bar=0.2 mm); D, Outer stratified squamous epithelia of the ventral region. Alcian blue (pH 2.5) (bar=25 μm); E, Outer stratified squamous epithelia of plug structure in the ventral region, Masson trichrome stain (bar=0.1 mm). (Abbreviations: ct, connective tissue; io, inner ovipositor; lm, lumen; mf, mucosal fold; ml, muscular layer; p, plug structure; rm, rectum; sel, squamous epithelial cell; arrowheads, mucous cell; 1, mucosa; 2, lamina propria-submucosa; 3, a circular inner muscle layer; 4, a longitudinal outer muscle layer; 5, serosa surrounded by stratified squamous cells.

swollen ventral process as a tube (Fig. 3A, 3D; Fig. 4A and 4B). The rectum consisted of four layers: mucosa, lamina propria-submucosa, muscularis, and squamous epithelial layer (peritoneum=serosa) (Fig. 3B).

The mucosa is composed of 4 cells such as columnar epithelial cells, mucus-secreting cells, lymphocytes, and granular cell. The columnar epithelial cells were tall and cylindrical with oval nuclei situated either centrally or toward the base of the cells (Fig. 4A and 4B). The mucous cells were spherical, flask-shaped, or ovoid, and interspersed among the columnar epithelial cells (Fig. 4A and 4B). The cells were highly vacuolated

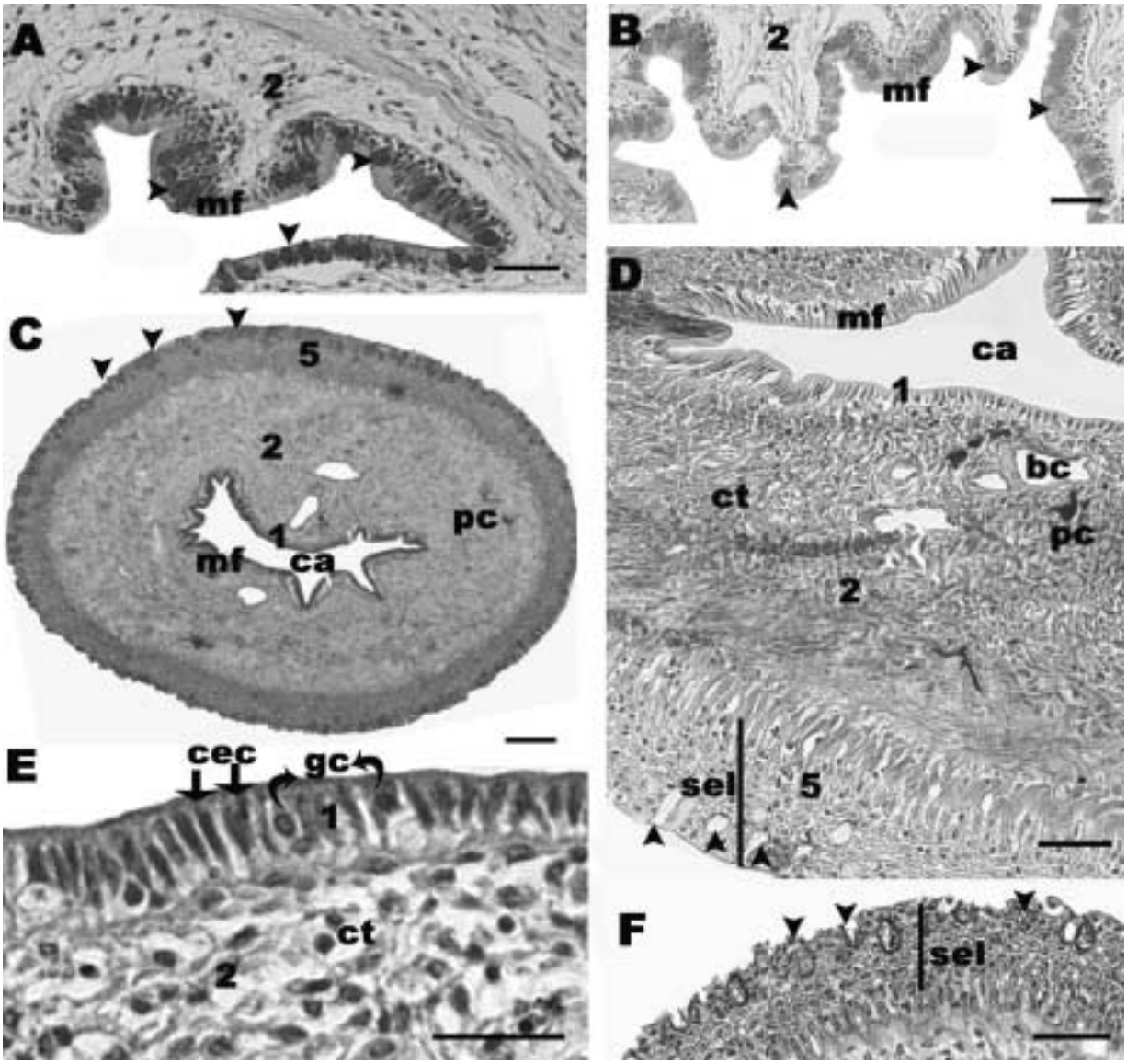

Fig. 4. Transverse section of the ventral process and ovipositor of *Acheilognathus koreensis*. A, Mucous cell stained with PAS reaction in the rectum (bar=10 μm); B, Mucous cell stained with AB (1.0) in the rectum (bar=10 μm); C, Overall structure of the outer ovipositor. Ehrlich haematoxylin and eosin (bar=0.1 mm); D, Structure showing three layers of the outer ovipositor. Masson trichrome stain (bar=10 μm). E, Mucosa and lamina propria-submucosa of the outer ovipositor. Ehrlich haematoxylin and eosin (bar=20 μm); F, Serosa surrounding by stratified squamous epithelia in the outer ovipositor. Ehrlich haematoxylin and eosin (bar=40 μm). (Abbreviations: bc, blood capillary; ca, cavity; cec, columnar epithelial cell; ct, connective tissue; gc, ganulocyte; mf, mucosal fold; pc, pigment cell; sel, squamous epithelial cell; arrowheads, mucous cell; 1, mucosa; 2, lamina propria-submucosa; 5, serosa surrounded by stratified squamous cells.

and basophilic haematoxylin and eosin preparations. The mucous cells are positive with PAS, AB at pH 1.0 and 2.5, indicating the presence of acidic mucopolysaccharides (Fig. 4A and 4B). The mucosa has longitudinal folds consisting of the lamina propria-submucosa and the lining epithelium along the entire length of the rectum (Fig. 3B; Fig. 4A and 4B). These folds close to the anus became low and slightly wavy to straight. Lymphocytes surrounded by small amounts of faintly stained cytoplasm are seen. Granular cells are seen in the mucosa and lamina propria-submucosa, but less in the muscularis and serosa.

The lamina propria-submucosa lying below the

mucosa consisted of a lamina propria and submucosa with no intervening muscularis mucosa (Fig. 3B; Fig. 4A and 4B).The lamina propria contained thin collagen bundles in a mesh-like appearance and extended into the mucosal folds. The submucosa is composed of a stratum compactum lying between the lamina propria and the inner circular muscle layer of the muscularis (Fig. 3B). The stratum compactum consisted of bundles of coarse and uniform collagenous fibers which were arranged compactly in several layers. This layer has granular cells, lymphocytes, blood vessels and nerve cells (Fig. 3B).

The muscularis under the lamina propria-submucosa has a circular inner layer and a thinner longitudinal outer layer (Fig. 3B). Between two the muscle layers are small amounts of connective tissue containing blood vessels and fibers. In particular, the muscularis in the body of the ventral process toward the body cavity is a thicker longitudinal layer, but in its the opening (vent) terminal region become thinned, a thinner, longitudinal layer.

The serosa peripheral to a the muscularis was consisted of connective tissue layer covered by stratified squamous cells (Fig. 3B and 3C).

(3) Inner ovipositor

The inner ovipositor is a tubular structure situated behind the rectum in the ventral process as a tube (Fig. 2). The inner ovipositor consisted of four layers: mucousa, lamina propria-submucosa, mucularies and squamous epithelial layer (peritoneum=serosa) (Fig. 3A and 3C).

The mucousa has a single layer of columnar epithelial cells with granular cells. Unlike the rectum, the epidermis has no mucous cell. The simple columnar epithelium that lines longitudinal lumens is tall in the body cavity and shortens as the inner ovipositor reaches outer ovipositor. The columnar epithelial cells are tall and cylindrical with oval nuclei situated either centrally or toward the base of the cells. Lymphocytes surrounded by small amounts of faintly stained cytoplasm are seen. There are granular cells in the epidermis.

The lamina propria-submucosa is separated by the basement membrane of the epithelial layer. This layer consisted of a lamina propria and submucosa with no intervening muscularis mucosa. The lamina propria consisted of thin collagen bundles in a mesh-like appearance and extended into the mucosal folds. The submucosa has a stratum compactum which is composed of bundles of coarse and uniform collagenous fiber which were arranged compactly in several layers. This layer has pigment cells, blood vessels and nerve cells.

The muscularis is composed of an inner circular or spiral layer and outer longitudinal layer. But the boundary between these layers is often indistinct. The loose connective tissue also fills spaces the bundles of muscles. This layer is thickest toward the body cavity but thinner in the end of the ventral process. The squamous epithelia provides the serosal covering of the inner oviduct.

2) Outer ovipositor

The outer ovipositor is a long and slender tube without the ventral process (Fig. 2). The outer ovipositor is similar to the inner ovipositor but simple than the inner ovipositor. The outer ovipositor consisted of three layers: mucousa, lamina propria-submucoa, and squamous epithelial layer (peritoneum=serosa) (Fig. 4C to 4F).

The mucosa is characterized by several longitudinal folds (Fig. 4C and 4D). These folds are lowered as it reaches to the opening of the outer ovipositor. A single of columnar epithelium is present along the folds and there are lymphocytes between epithelial cells has no mucous cell (Fig. 4E and 4F)

The lamina propria-submucoa consisted of a lamina propria and submucosa with no intervening muscularis mucosa (Fig. 4D to 4E).The lamina propria has irregular, loose, and thin collagen bundles and the submucosa have a stratum compactum consisted of collagen bundles which are arranged densely in parallel or organized fashion (Fig. 4D to 4E). The thickness is reduced as it reach to the end of outer ovipositor. There are pigment cells, blood capillaries and nerve cells.

The muscularis was not present in the outer ovipositor (Fig. 4C and 4D). The squamous epithelia, serosa, circumscribes the outer ovipositor.

Discussion

In most bony fishes, the anus or vent is on the mid-ventral line of the body and the second half of the over-all length of the individual, behind the bases of the pelvic fins and just in front of the anal fin. In this case, the opening of the urogenital duct which contains the exits of the uri-

nary and genital ducts are at the surface, behind the anus (Lagler *et al*., 1977), A few accessory reproductive structures among females serve as sexual characteristics. Some fishes as bitterling, lumpsucker and oily shiner fishes have the egg-laying tube or ovipositor in the female for spawning season (Lagler *et al*., 1977; Kim and Kim, 1990, 1991; Suzuki and Jeon, 1991; Song and Kwon, 1994; Kim and Yang, 1998; Arai *et al*., 2001; Kim and Park, 2002; Baek *et al*., 2002, 2003; Baek and Song, 2005; Kim *et al*., 2005; Song and Son, 2005). During the spawning season in the bittering, subfamily Acheilognathinae, the female possesses a long ovipositor behind its anal opening that they use to place their eggs onto the gills of a mussel (Heschl, 1989; Aldridge, 1999; Candolin and Reynolds, 2001; Mills and Reynolds, 2003; Smith *et al*., 2004; Kim *et al*., 2005). As the bittering reaches spawning season, the ventral region is protruded from the body surface and then lengthen outward. Finally the female of the bittering has a ventral process with a long and slender tract.

During spawning season of a Korean oily bitterling the female's ventral region near the base of the pectoral fin becomes to be protruded outward of body and enlarged. Through the histological approach on the ventral process, it had known that the ventral process consisted of both organs as rectum and inner ovipositor. In the ventral region, the rectum consisted of mucosa, lamina propria-submucosa, muscularis, and squamous epithelia (peritoneum=serosa) and the mucosa epithelium had mucous cells meaning acid mucopolysaccharides in nature as reported in other bony fishes (Bullock, 1967; Reifel and Travill, 1979; Anderson 1986; McLeese and Moon 1989; Williams and Nickol 1989; Tibbetts 1997; Park, 2001; Park *et al*., 2003). And, the structure of the inner ovipositor was similar to that of the rectum as it consists of a mucosa, lamina propria-submucosa (submucosa), muscularis and serosa. However, there was a clear difference between the rectum and inner ovipositor. It was that the mucosa layer in the inner ovipositor had no mucous cell unlike that of rectum. The presence of the mucous cell in the rectum serves a digestive function. The mucus secreted by mucous cells aids lubrication of the food during passage and may provide cofactors required for enzymatic degradation of the food (Anderson, 1986).

The outer ovipositor is a straight and long tube which are not connected with the ventral process any more. The outer ovipositor was similar to the structure of the inner ovipositor in the ventral process. However, interestingly, there was a difference between the inner ovipositor and the outer ovipositor. The outer ovipositor had no muscularis and consisted of three layers such as a mucousa, lamina propria-submucoa, and squamous epithelia. The presence of the muscularis in the inner ovipositor may be related with rhythmic contractions of the layers of the muscularies for propelling to the matured oocytes to the outer ovipositor, finally for laying eggs inside mussels, as in peristaltic movement of intestine with well-developed muscularies. Whereas, the absence of the muscularis in the outer ovipositor seems to be just tube that eggs discharged from the outer ovipositor allow to send inside mussel. Probably, it means that energy for moving the eggs to mussel is not results from outer ovipositor but rhythmic contractions of the well-developed muscularis in the inner ovipositor of the ventral process. Therefore, the outer ovipositor serves as only a transmitting tube.

Multi-layered squamous epidermal cells in ventral process, inner and outer ovipositor were present, and it was giving a serosa, situated at the outermost layer of the rectum and the inner ovipositor. In bony fishes, a serosa of the intestine consisted generally of a thin layer of connective tissue covered by a single layer of squamous cells (Bullock, 1967; Reifel and Travill, 1979; Anderson, 1986; McLeese and Moon, 1989; Tibbetts, 1997; Park, 2001; Park *et al*., 2003). But present of the multi-layered sqamous epidermal cells is related with cell proliferation that the ventral process and ovipositor are protruded out of the body as the spawning season of the female comes up.

In the outermost region of the squamous epidermal layer, numerous exocrine unicellular mucous cells of acidic mucopolysaccharides are present. Generally these mucous cells are common to most fishes and occurs in the internal epithelial of fishes (Mittal and Munshi, 1971; Mittal and Banerjee 1974; Mittal *et al*., 1980; Whitear, 1986), The mucus in the epidermis serves protective functions as vaccines to bacteria and antigen, or protection against abrasion (Hockey, 1985).

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산란시기의 칼납자루 *Acheilognathus koreensis* (잉어과)의 배측융기와 산란관의 구조

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한국산 칼납자루의 암컷은 산란시기가 되면 배지느러미 기부 부근에 돌출되어 신장되어 나타나는 배측융기가 형성된다. 이러한 배측융기는 직장(항문)부분과 내측산란관으로 구성되었다. 직장은 점막층, 점막하층, 근육층과 이들을 둘러싸는 다층의 표층으로 구성되어 있으며 특히 점막층에는 산성 점액다당류인 점액세포가 존재한다. 한편 내측산란관은 직장과 비슷한 구조를 가지고 있으나 점막층에는 점액세포가 존재하지 않는 특징을 보였다. 한편 외측산란관은 배측융기와 연결되지 않아 가늘고, 긴 관의 형태를 보이고 있으나 내측산란관과 달리 근육층이 존재하지 않아 점막층, 점막하층, 다층의 표피층으로 구성되어 있다. 이러한 결과로 보아 근육층을 가지는 내측산란관은 성숙란을 외측산란관으로 내보기 위해 연동운동과 관련있는 반면에 외측산란관은 근육층이 존재하지 않아 내측산란관으로부터 방출된 성숙란을 단지 이매패 속으로 전달하는 단순한 관의 역할을 하는 것으로 생각된다.