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# Study on the Pigmentation of Albinic Bitterlings *Acheilognathus signifer* (Pisces; Cyprinidae) Based on Its Entire Body, Appendage and Eye

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**ABSTRACT** During an artificial breeding as a part of restoration of the endangered Korean bitterling *Acheilognathus signifer*, a small number of individuals exhibiting oculocutaneous albinism were produced. We compared the pigmentation and morphology of normal and albinic bitterlings by histological examination of skin samples obtained from 10 regions on the body, fins, and eyes. There were no differences in morphometry and in general morphology of skin between them. In normal bitterlings, pigment cells were better developed in the dorsal region, the upper part of caudal peduncle region, the choroid-retinal epithelium and iris than in other areas. In the albinic bitterling, however, pigment cells were present only in three parts of the dorsal region, the caudal and dorsal fin, which had few melanin cells. Albinic bitterlings also displayed deficient pigmentation in the choroid-retina pigment epithelium and iris. Although they had different pigmentation aspects in distribution and development between normal and albinic bitterlings, melanin cells were mainly present in the dorsal regions of the skin and eyes where are exposed directly to light.

**Key words** : Korean bitterling, *Acheilognathus signifer*, oculocutaneous albinism, pigment cells

## INTRODUCTION

The Korean bitterling *Acheilognathus signifer* is designated as an endangered freshwater fish by the Ministry of Environment of Korea (2005) due to its decreased distribution and deteriorating aquatic environment. Since 2006, there has been an effort by National Fisheries Research & Development Institute (NFRDI) to preserve this species through artificial breeding. During the artificial breeding, albinic bitterlings showing phenotypically conspicuous oculocutaneous albinism emerged. Albinism, which may be caused by negative regulation of pigmentation (Lin and Fisher, 2007), has been known to occur across taxa including fish (Vielkind *et al.*, 1971; Koga and Hori, 1997; Fukamachi *et al.*, 2001; Okulicz *et al.*, 2003; Yoo *et al.*, 2003; Lamoreux *et al.*, 2005; Gronskov *et al.*, 2007; Kang *et al.*, 2007). However the mechanisms responsible for albinism are still remained unclear.

A histological study was recently conducted to investigate the occurrence of oculocutaneous albinism (OCA) in this species (Oh *et al.*, 2008), but it considered only one region of dorsal skin and eyes for pigmentation analysis and even not suggested whether there were any morphometric differences between the albinic and normal bitterlings. As the melanin pigment is considered what it is responsible to the light (Bolker and Hill, 2000), it is questionable whether the melanin pigment is restricted in the skin where are directly exposed to sunlight or distributed in overall skin region in both normal and albinic bitterlings. Moreover it is still wondered if there are any differences in morphometry and malanin distribution patterns. So we examined multiple regions including the skin, appendages and eyes of this species for the morphological and histological study.

## MATERIALS AND METHODS

Ten each specimens of laboratory-reared normal and albinic bitterlings were examined in morphometric analy-

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sis and histological study. Method for proportional measurements and counts for morphometry was followed by the Hubbs and Lagler (2004). For histological study, samples were taken from the skin in five regions (dorsal, lateral, ventral, caudal peduncle, and occiput region), from three fins (dorsal, anal and caudal fin) and from the choroid-retina and the iris (Fig. 1). Samples of skin tissue were gradually dehydrated by a standard ethanol series, cleared in xylene, and embedded in paraplast. The 5 μm sectioned preparations were deparaffinized in xylene and stained with hematoxylin-eosin for assessment of general structure and with Fontana-Masson for detection of argentaffin melanin cells. Tissue microstructure was analyzed using an Axio imager A1 microscope (Carl Zeiss, Germany) and the Axio Vision (Ver. 4.5, Germany).

We used a transmission electron microscope (TEM) to visualize the ultrastructure of melanin pigment cells. Skin samples were fixed in 2.5% glutaraldehyde and then in 1% osmium tetroxide. After dehydration in a graded alcohol series, tissues were embedded in Epon 812 resin. Ultrathin samples of 100 nm thick were stained with uranyl acetate and lead citrate to enhance electron density, and observed with an EOL-1200EX TEM.

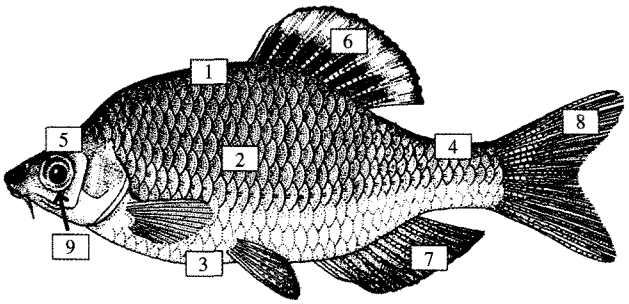


**Fig. 1.** Sampling regions of body skin, appendages, and eyes of the Korean bitterling, *Acheilognathus signifer*. (1) dorsum, (2) lateral region, (3) ventral region, (4) caudal peduncle region, (5) occiput, (6) dorsal fin, (7) anal fin, (8) caudal fin, (9) eyeball.

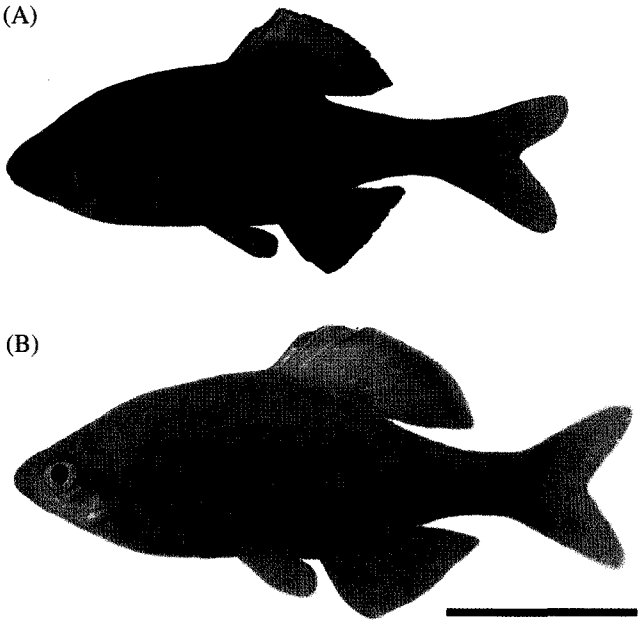


**Fig. 2.** The external morphology and coloration of the Korean bitterling. (A) normal bitterling; (B) albino bitterling. Bar indicates 2 cm.

## RESULTS

### 1. Color pattern and morphometry in the normal and albinic group

The body color of normal bitterlings was dark green with the marginal region of the dorsal fin and the basal region of the caudal fin appearing yellow. Black shading was dispersed over the entire body, the dorsal fin, and

**Table 1.** Comparison of proportional measurements and counts between normal and albinic bitterling, *Acheilognathus signifer*

| Characters | Present study | | Kim (1997) |
|---|---|---|---|
| | Normal (n=10) | Albino (n=10) | |
| Standard Length (SL, mm) | 36.1~45.1 | 32.9~56.9 | |
| **In SL** | | | |
| Head length (HL) | 22.8~25.4 (24.2±1.0) | 23.7~26.4 (25.1±1.2) | 22.5~27.5 |
| Body depth | 29.9~36.8 (32.3±3.1) | 32.5~38.8 (34.7±2.8) | 35.7~50.9 |
| Length of caudal peduncle | 22.2~26.8 (24.7±2.0) | 23.7~25.1 (24.5±0.7) | 15.9~22.0 |
| Depth of caudal peduncle | 10.8~13.3 (12.0±1.0) | 11.7~13.7 (12.9±0.9) | 11.7~13.9 |
| Predorsal length | 53.2~54.6 (54.1±0.6) | 52.6~55.1 (54.4±1.2) | 50.7~57.2 |
| **In HL** | | | |
| Snout length | 24.1~32.8 (29.5±3.8) | 25.2~28.4 (26.9±1.5) | 27.5~37.1 |
| Interorbital width | 33.9~40.3 (36.4±2.7) | 31.7~38.3 (34.4±3.2) | 32.3~41.3 |
| Orbit diamiter (OD) | 33.0~37.9 (35.8±2.2) | 32.6~35.6 (33.6±1.4) | 25.9~35.5 |
| Barbel length | 10.7~20.4 (14.6±4.2) | 8.4~23.0 (14.7±6.4) | 17.1~28.7 |
| **In OD** | | | |
| Interorbital width | 0.9~1.2 (1.0±0.1) | 0.9~1.1 (1.0±0.1) | |
| Barbel length | 0.3~0.6 (0.4±0.1) | 0.3~0.6 (0.4±0.2) | |
| **Counts** | | | |
| Dorsal fin rays | iii, 8 | iii, 8 | iii, 8~9 |
| Anal fin rays | iii, 8~9 | iii, 7~9 | iii, 8~10 |
| Lateral line scales | 34~35 | 33~34 | 35~38 |
| Scales above lateral line | 6 | 5~6 | |
| Scales below lateral line | 4 | 4 | |
| Vertebrae | 35~36 | 35 | 31~34 |
| Gill raker | 7~9 | 7~8 | 7~8 |

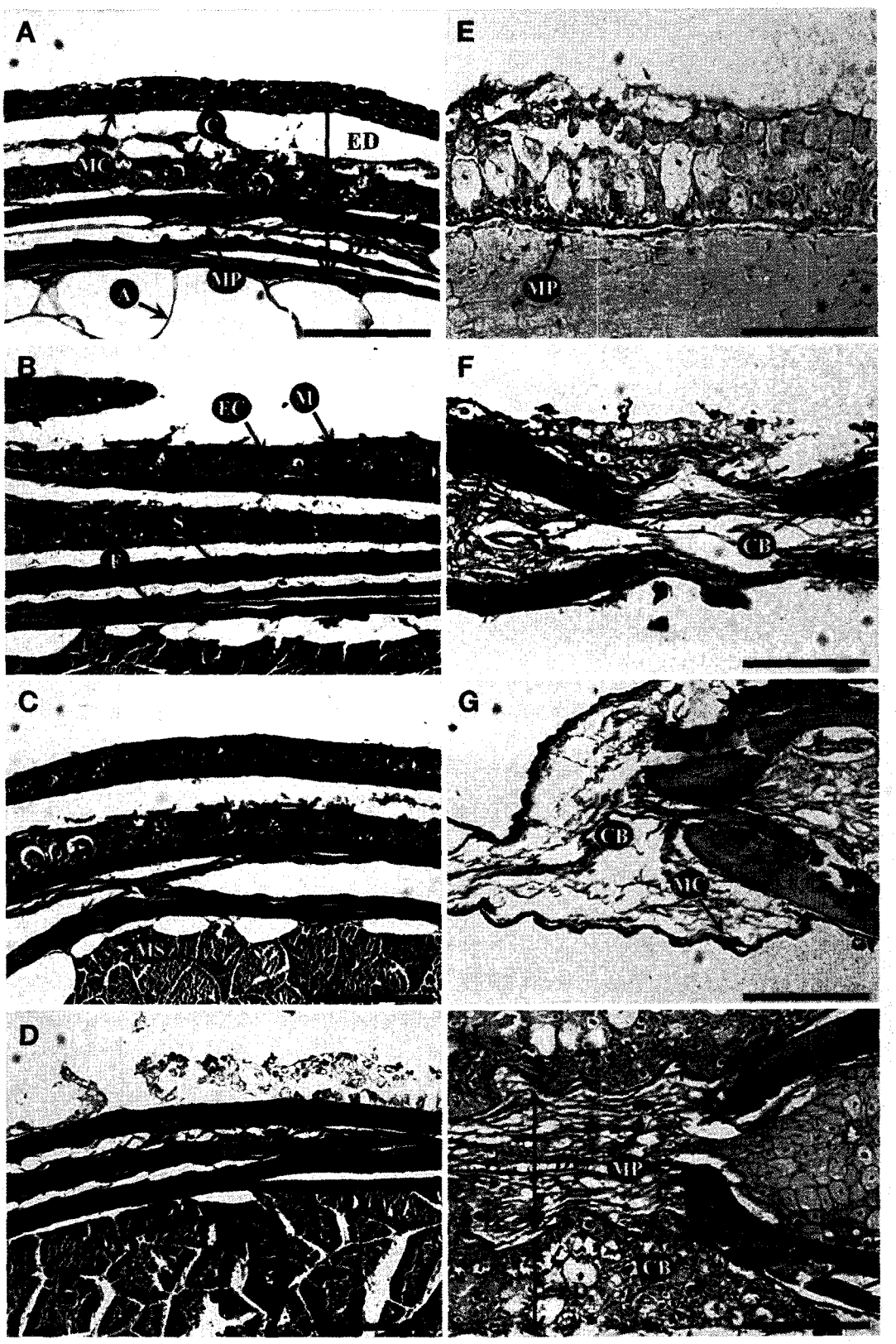


**Fig. 3.** General morphology of the 8 regional skins in the normal bitterling . (A) dorsal region, (B) lateral region, (C) ventral region, (D) caudal peduncle region, (E) occiput, (F) dorsal fin, (G) anal fin, (H) caudal fin. A, adipose cell; C, club cell; CB, cartilage bone; DE, dermis; EC, epithelium cell; ED, epidermis; F, fibroblast; M, mucus cell; MC, melanocyte; MP, melanophore; MS, muscle; S, scale; SC, subcutis. Bars indicate 100 μm. *Hematoxylin-Eosin staining.*

the anal fin. The eyes were conspicuously black (Fig. 2A). In contrast, the skin of albino bitterlings appeared yellow-white over the entire body. The marginal regions of the dorsal fin and the caudal fin base were yellow as in normal bitterlings. The eyes were tinged with red (Fig. 2B). There were no morphometric differences between normal and albinic bitterlings (Table 1). The values of measurements and counts were fully overlapped for every characteristics, which values corresponded well with data from wild type bitterlings (Kim 1997).

### 2. General morphology in histological observation

#### 1) Skin

The skin of the normal and albinic bitterlings was composed of epidermis, dermis and subcutis (Fig. 3). The epidermis consisted of stratified squamous epithelium cells in about six rows and has two types of glandular cells, mucus and large club cells. Pigment cells were positioned just beneath or in the epidermis. Pigment cells were easily visualized as dark brown granule-packed structures under a light microscope, but under TEM they were revealed to be aggregations of melanosomes within the cytoplasm, known as melanocytes (Fig. 4A). The dermis consisted of dense connective tissue with collagen fibers and fibroblast cells. Occipital skin samples had very thin dermal layers, which distinguished them from skin samples taken from other regions of the body. A lot of blood capillaries were to be seen beneath the epidermis and melanophores, electron-opaque ellip-

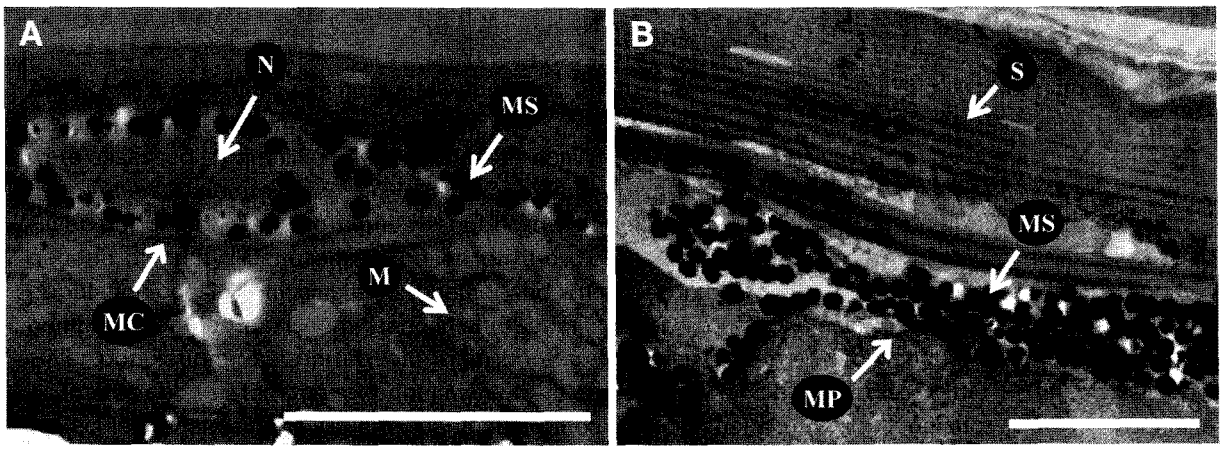


**Fig. 4.** TEM showing the distribution of melanosomes in epidermis (A) and dermis (B) layers. Melanocytes and melanophores located above and below scale, respectively. M, mucus cell; MC, melanocyte; MP, melanophore; MS, melanosome; N, nucleus of melanocyte; S, scale. Bar indicates 5 μm.

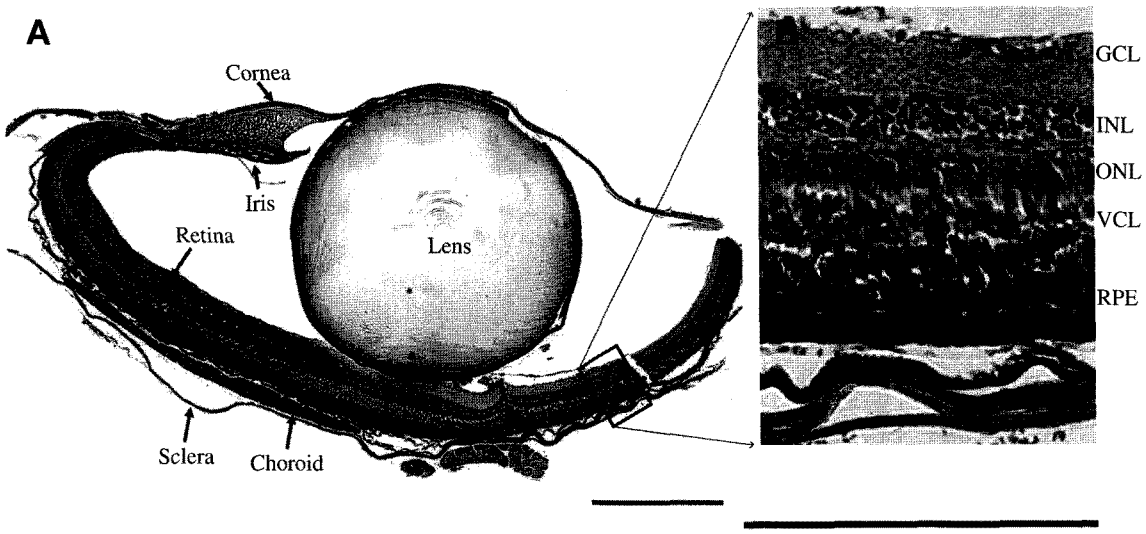


**Fig. 5.** The radial section of the eyeball (A) and enlargement of the retina (B) in the normal bitterlings. RPE, retina pigment epithelium; VCL, visual cell layer; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Bars indicate 500μm. Hematoxylin-Eosin staining.

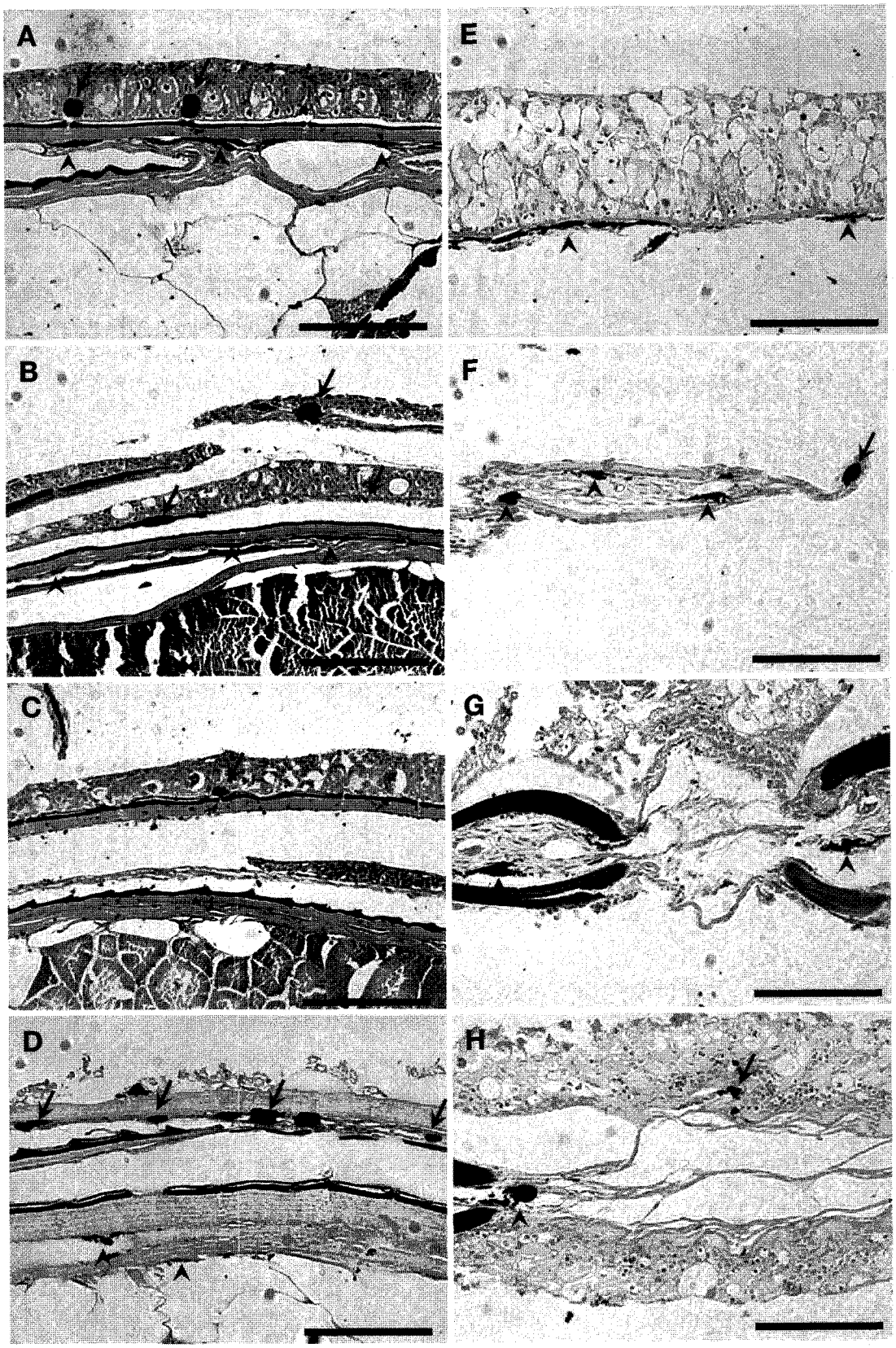


**Fig. 6.** Distribution of melanocytes and melanophores in the 8 regional skins of normal bitterlings. (A) dorsal region, (B) lateral region, (C) ventral region, (D) caudal peduncle region, (E) occiput, (F) dorsal fin, (G) anal fin, (H) caudal fin. The pigment cells represent black as a result of accumulation of argent anion. Arrows, melanocytes; arrowhead, melanophores. Bars indicate 100 μm. Fontana-Masson staining.

**Table 2.** Number of the melanocyte and melanophore distributed over the epidermis and dermis layer per 1 mm length in the normal and albinic bitterlings, *A. signifer*

| | Normal | | Albino | |
|---|---|---|---|---|
| | Melanocyte | Melanophore | Melanocyte | Melanophore |
| Dorsal region | 8~10 (9.3±1.2) | 8~18 (14.0±5.3) | 0~1 (0.8±0.4) | 0~2 (1.4±0.5) |
| Lateral region | 2~4 (2.7±1.2) | 2~3 (2.7±0.6) | – | – |
| Ventral region | 1~2 (1.5±0.7) | 1~3 (2.5±0.7) | – | – |
| Caudal peduncle region | 3~5 (4.0±1.4) | 12~17 (14.5±3.5) | – | – |
| Occiput | 2~6 (3.3±2.3) | 4~6 (6.0±2.0) | – | – |
| Dorsal fin | 7~9 (8.0±1.4) | 4~5 (4.3±0.5) | 1~2 (1.0±0.7) | 0~1 (0.8±0.4) |
| Anal fin | 6~8 (7.0±1.4) | 2~4 (2.8±0.8) | – | – |
| Caudal fin | 5~9 (7.0±2.8) | 3~4 (3.6±0.3) | 1~2 (1.2±0.4) | 0~1 (0.6±0.5) |

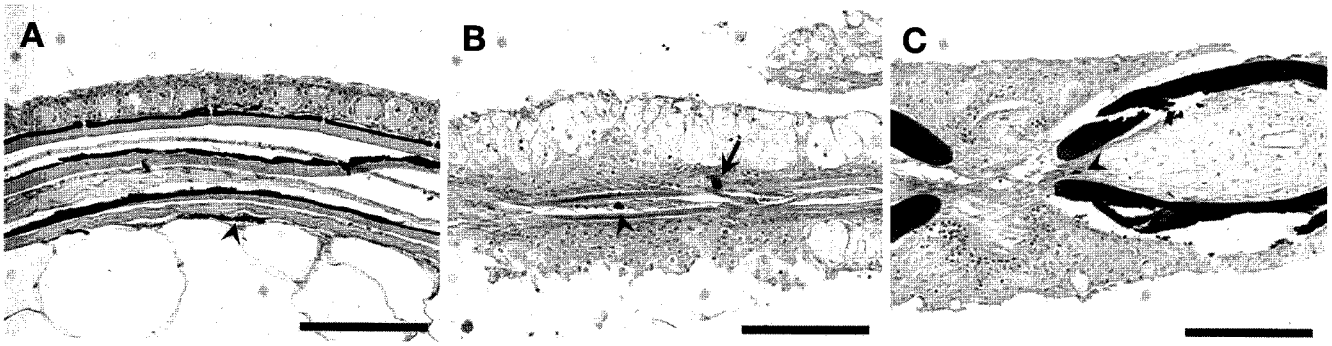


**Fig. 7.** Distribution of melanocytes and melanophores in albinic bitterling. Pigmentation only occurred in the dorsum (A), dorsal fin (B), and anal fin (C) region. The pigment cells are stained with black as a result of accumulation of argent anion. Arrows, melanocytes; arrowhead, melanophores. Bars indicate 100 μm. Fontana-Masson staining.

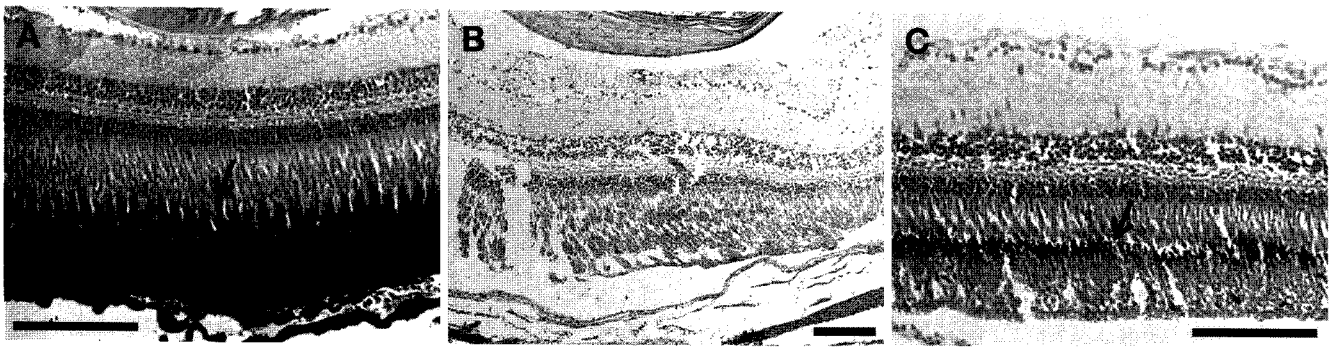


**Fig. 8.** Choroid-retina pigment epithelium of normal (A) and albinic (B and C) bitterlings. Arrows represent accumulated melanin pigment cells. Bars indicate 100 μm. Fontana-Masson staining.

soidal structure, were dispersed around (Fig. 4B). The subcutis was confined between the dermis and the muscle, and composed mainly of blood vessels and large adipose cells shaping empty space.

#### 2) Eyes

The eyes were composed of lens, sclera, cornea, iris, choroid, and retina (Fig. 5A). The retina consisted of a stratified layer: retina pigment epithelium, visual cell layer, outer nuclear layer, inner nuclear layer and ganglion cell layer (Fig. 5B). In the retina pigment epithelium region, the pigment was well developed in the normal group but it was very little in the albinic group. The basic structure of the eyes was the same in the albinic and normal groups.

### 3. Pigmentation of the skin, appendages, and eyes

#### 1) Regional body skin and appendages

The pigment cells consisted of epidermal melanocytes

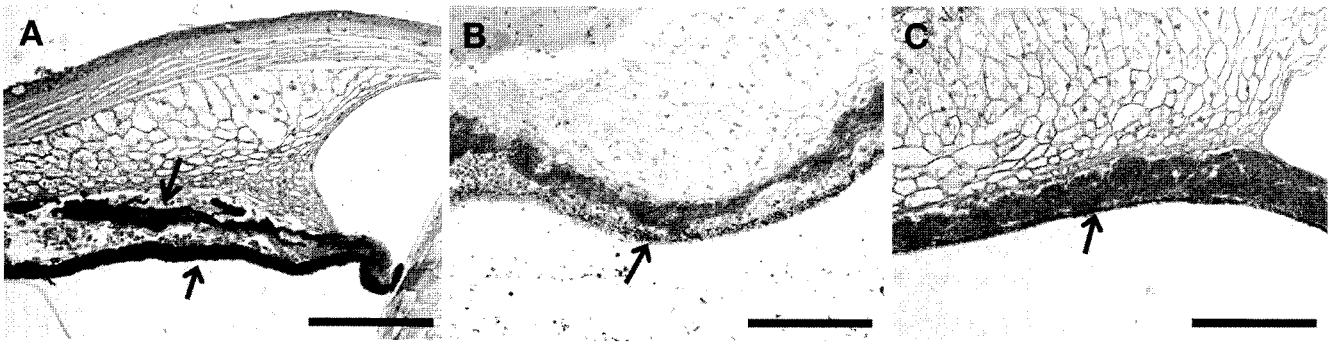


**Fig. 9.** Iris of normal (A) and albinic bitterling (B and C). Black regions (arrows) represent melanin pigmentation sites. Bars indicate 100 μm. Fontana-Masson staining.

and dermal melanophores. The number of the melanocytes and melanophores in normal bitterling was the greatest in the dorsal region, about 9.3±1.2 and 10.0±5.3 per 1 mm length, respectively. The upper part of caudal peduncle was about 4.0±1.4 of melanocytes and 14.5±3.5 of melanophores. The ventral and lateral regions contained fewer cells, about 1.5±0.7 and 2.7±1.2 of melanocytes and 2.5±0.7 and 2.7±0.6 of melanophores, respectively (Table 2; Fig. 6). Samples from the lateral, ventral, caudal peduncle region, occiput, and anal fin of albinic bitterlings exhibited no pigment cells. However, pigment cells were found in small numbers in the dorsal and anal regions, and caudal fin of albinic bitterling, 0~2 for both melanocytes and melanophores (Table 2; Fig. 7).

#### 2) Choroid-retina pigment epithelium and iris

In the normal group, pigment cells were abundant throughout the choroid-retina pigment epithelium (Fig. 8A), whereas in the albinic group they were absent (Fig. 8B) or found only in low quantities (Fig. 8C). The iris epithelium of normal bitterling had abundant pigment cells (Fig. 9A), but it showed vestigial distribution in the albinic group (Fig. 9B and 9C).

## DISCUSSION

The phenomenon of oculocutaneous albinism is well known as a consequence negative regulation of pigmentation, for which the mechanism is unclear (Lin and Fisher, 2007). It is sometimes caused by a genetic disorder in the melanin synthesis pathway (Gronskov *et al.*, 2007). Many studies of OCA focused on its clinical relevance in humans (Oetting *et al.*, 2003; Lin and Fisher, 2007) or on the incidence of albinism in commercially important fish species (Yoo *et al.*, 2003; Kang *et al.*, 2007). A number of experiments have been conducted to determine why this phenomenon occurs in nature, which revealed that nutrition, lighting, and substrates are all important influences (Bolker and Hill, 2000; Kang *et al.*, 2007). What we could safely surmise for the albinism through the experiment in this paper was that the albinism might be the result of a recessive allele, because of all the albinic bitterlings that we examined were the offsprings of two of phenotypically identical albinic parents.

Albinism can be classified into 4 types of OCA1, OCA2, OCA3, OCA4 (Okulicz *et al.*, 2002; Gronskov *et al.*, 2007) according to skin coloration, and 2 types of $i^1/i^1$ and $i^4/i^4$ (Koga and Hori, 1997) according to eye coloration. From the classifications for albinism, there was no question that the albinic Korean bitterling should be categorized as OCA1 and both $i^1/i^1$ and $i^4/i^4$ (quasi-albino), due to the presence of yellowish white skin and red eyes. However the $i^1/i^1$ phenotype was more common (80%) than $i^4/i^4$ (20%).

Interestingly, the dorsal and the upper caudal peduncle region in normal bitterling had many more pigment cells than the other regions of the body (Table 2). These differences may be explained by the different levels of light exposure experienced by dorsal and ventral surfaces of the body (Bolker and Hill, 2000). The few pigment cells in albinic bitterling were restricted to the upper regions of the dorsal skin, dorsal fin, and caudal fin, where direct exposure to light occurs. To better understand the correlation between pigmentation and light, physiological approaches using light-controlled experiments should be warranted.

Pigment cells were abundant in the choroid-retina pigment epithelium and iris of normal bitterling, but scarce in albino one, consistent with the findings of previous studies (Oh *et al.*, 2008). However, we found that pigment cells were located in both inner and outer sides of the iris in normal bitterling, but restricted to the inner side in albinic bitterling (Fig. 9). Melanin cells in the choroid-retina pigment epithelium were overspread in normal bitterling, but restricted to a narrow upper region in albinic one (Fig. 8). We conclude that albinic bitterling are not truly melanin deficient, and that levels of pigmentation may be related to light exposure. We do not know, however, whether pigmentation is a result of active or passive

reactions to light for now.

## ACKNOWLEDGMENTS

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# 알비노 묵납자루의 부위별 색소발현에 관한 연구

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**요 약 :** 멸종위기종인 묵납자루 *Acheilognathus signifer*의 종복원을 위한 인공수정을 실시하는 과정에서 눈과 피부의 색소발현이 결여된 백색증 개체가 출현하였다. 정상 묵납자루와 백색증 개체간 색소발현과 형태의 차이 여부를 알아보기 위하여 몸통, 지느러미, 눈 등 총 10개 부위에 대한 조직학적 검사를 실시하였다. 그 결과 정상 묵납자루의 경우, 멜라닌세포는 빛에 쉽게 노출되는 등부위와 상미병부, 맥락막-망막색소상피층 및 홍채에서 다량으로 분포하였다. 반면에 백색증 개체에서는 멜라닌세포가 등 부위와 등지느러미, 그리고 꼬리지느러미에서 아주 소량으로 분포하고 있었으며 눈의 맥락막-망막색소상피층 및 홍채에서는 색소결핍 현상이 뚜렷하게 관찰되었다.

**찾아보기 낱말 :** 묵납자루, *Acheilognathus signifer*, 백색증, 멜라닌세포