Received: August 24, 2009 Revised: September 21, 2009 Accepted: September 25, 2009

Histological Study on Cutaneous Albinism of Korean Slender Gudgeon *Squalidus gracilis majimae* from Korea

By Yong-Joo Lee, Jong-Young Park^{1,*} and Min-Ki Oh¹

Jeonju National University of Eduaction, Jeonju 560-757, Korea
¹Faculty of Biological Sciences and Institute for Biodiversity Research, College of Natural Sciences, Chonbuk National University, Jeonju 561-756, Korea

ABSTRACT Histological examination of an individual of the Korean slender gudgeon *Squalidus gracilis majimae* having cutaneous albinism produced rarely in the wild was made and compared with a normal individual. The external body of the albino was colorless, differing from the normal individual, which has dense brownish black spots over its body surface. To make it clear through histological study, we observed eight skin regions: dorsal, lateral, ventral, upper caudal peduncle, lower caudal peduncle, dorsal fin, anal fin, and the eyes. These regional skins were the same in fundamental structure between albinic and normal gudgeon, but there were significant differences in distribution and development of pigment cells (melanins). In the normal gudgeon, the pigment cells were well developed over the regional skins except on the skin from the ventral region. However, it was confirmed in the albino that the pigment cells were vestigial over the upper regions of the eye and body but absent in the ventral region, lower caudal peduncle, and anal fin.

Key words: Squalidus gracilis majimae, cutaneous albinism, pigment cell, melanin

INTRODUCTION

The Korean slender gudgeon, Squalidus gracilis majimae is an endemic subspecies having wide geographical distribution in Korea (Kim et al., 2005). During a survey for fish fauna in Nakdong River in May 9, 2008, one albinic gudgeon phenotypically presumed to be cutaneous albinism was collected. The phenomenon of albinism is supposed to the negative regulation of pigmentation (Lin and Fisher, 2007) by deficiency of melanin synthesis (Koga and Hori, 1997). In general, the pigment cells may be related with light in biological function (Bolker and Hill, 2000) and the occurrence of albinism could be explained by several causes as a genetic disorder (Gronskov et al., 2007), a transportable element (Fukamachi et al., 2001) or an acquired influences including nutrition, lighting and substrate (Bolker and Hill, 2000; Kang et al., 2007). Such phenomenon has been well known in many fishes including medaka Oryzias latipes (Fukamachi et al., 2001), flatfish Paralichthys olivaceus (Yoo et al., 2003; Kang et al., 2007), bitterling Acheilognathus signifer (Oh et al., 2008), catfish Silurus asotus (Park et al., 2009), mandarin fish Siniperca scherzeri (Lee et al., 1997). Most of them, however, occurred in the artificial environment for aquaculture or emerged from the genetic recombination for experimental purpose. Previous literatures concerning the slender gudgeon were dealt with ethology (Park et al., 2005a), embryology (Park et al., 2005b), taxonomic review (Kim and Lee, 1984) and chromosome analysis (Kim et al., 2004). Since the occurrence of the albinic gudgeon in the wild was not described until now, we are going to report it and investigate the histological differences between the normal and the albinic slender gudgeon, especially in the pigmentation from the body skin, the appendages and the eyes.

MATERIALS AND METHODS

One albinic and two normal specimens of Korean slender gudgeon collected from Nakdong River (Munam-ri, Eunchuk-myeon, Sangju-si, Gyungsangbuk-do, Korea) in May 9, 2008, were used in histological analysis to

^{*}Corresponding author: Jong-Young Park Tel: 82-63-270-3344, Fax: 82-63-270-3362, E-mail: Park7877@chonbuk.ac.kr

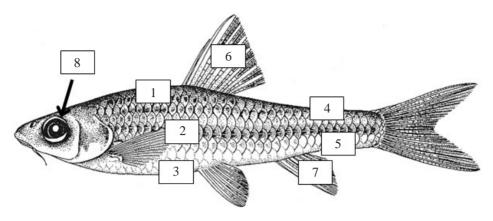


Fig. 1. Sampling regions for histological analysis of the Korean slender gudgeon, *S. gracilis majimae*. (1) dorsal region, (2) lateral region, (3) ventral region, (4) upper caudal peduncle region, (5) lower caudal peduncle region, (6) dorsal fin, (7) anal fin, (8) eyeball.



Fig. 2. External appearance of Korean slender gudgeon, S. gracilis majimae. (A) normal gudgeon, (B) albino gudgeon. Bar indicates 1 cm.

compare any differences in morphology and pigmentation between them. Tissue samples fixed with 10% neutral formalin solution were taken in five regions of the body (the dorsal, lateral, ventral, upper caudal peduncle and lower caudal peduncle region), two regions of the fins (dorsal and anal fin) and the eyes (Fig. 1). The tissues were dehydrated through standard ethanol series, cleared in xylene and embedded in wax (Paraplast, Oxford). The 5 µm sectioned preparations were deparaffinized in xylene and stained with hematoxylin-eosin (Presnell and Schreibman, 1997) for general structure and with Fontana-Masson (Jegal *et al.*, 2006) for argentaffin melanin cells.

Microstructure of tissues was analyzed under a microscope, Axio imager.A1 (Carl Zeiss, Germany) and the Axio Vision (Ver. 4.5, Germany).

RESULTS AND DISCUSSION

1. External appearance

The normal gudgeon showed white silver when it was alive, but mostly dark brown in a formalin solution-deposited condition (Fig. 2A). Moreover, the black spots were scattered over the overall body region, especially densely

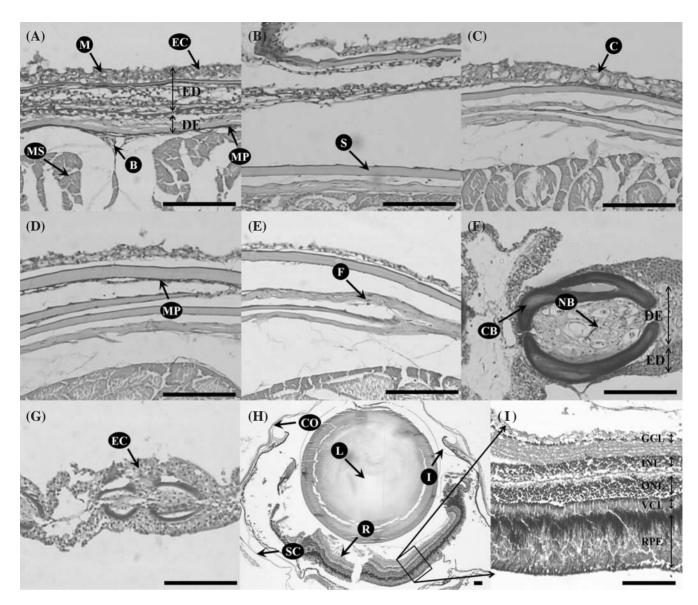


Fig. 3. General morphology on the eight regional skins of Korean slender gudgeon, *S. gracilis majimae*. (A) dorsal region, (B) lateral region, (C) ventral region, (D) upper caudal peduncle, (E) lower caudal peduncle, (F) dorsal fin, (G) anal fin and (H) the eyes. B: blood capillary, C: club cell, CB: cartilage bone, CO: cornea, DE: dermis, EC: Epithelium cell, ED: epidermis, F: fibroblast, GCL: ganglion cell layer, I: iris, INL: inner nuclear layer, L: lens, M: mucus cell, MP: melanophore, MS: muscle, NB: nerve bundle, ONL: outer nuclear layer, R: retina, RPE: retina pigment epithelium, S: scale, SC: sclera, VCL: visual cell layer. Bars indicate 100 μm. Hematoxylin-Eosin staining.

distributed along the whole lateral line scales. The appendages of the dorsal, the pelvic, the ventral, the anal, and the caudal fins were transparent and the eyes were tinged with absolutely black. However, the albinic gudgeon in the formalin solution was nearly bright color (Fig. 2B) and the black spots were very few or absent. All fins were transparent and the eyes were black which were the same as the normal one. It means that the albinic gudgeon may be the cutaneous albinism, differing from the oculocutaneous albinism of *Acheilognathus signifer* reported by Oh *et al.* (2008).

2. General morphology of skin and eye

The skin of the body trunk and the appendages in the normal and albinic gudgeon was mainly composed of epidermis and dermis (Fig. 3A~G). The epidermis consisted of predominant epithelial cells and comparatively large and specialized glandular cells, a mucus cell and a club cell. The basophilic mucus cells located at the upper region of the epidermis were not so enlarged when it compared with bottom-dwelling fishes such *Misgurnus anguillicaudatus* (Park and Kim, 1999; Oh and Park, 2008), *M. mizolepis* (Park *et al.*, 2001; Oh and Park,

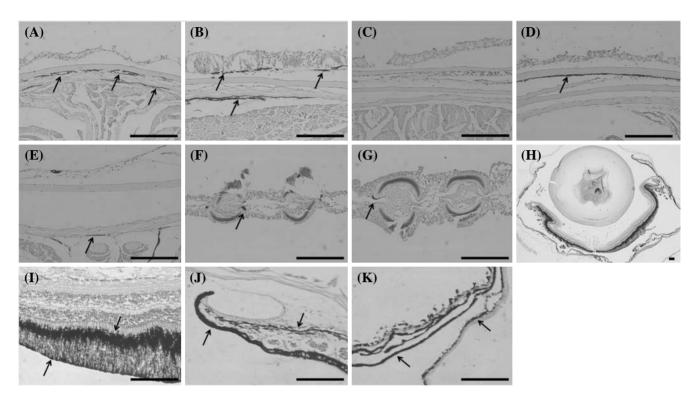


Fig. 4. Pigmentation of melanin cells on the eight regional skins in the normal Korean slender gudgeon, *S. gracilis majimae*. (A) dorsal region, (B) lateral region, (C) ventral region, (D) upper caudal peduncle, (E) lower caudal peduncle, (F) dorsal fin, (G) anal fin, (H) the eyes, (I) retina, (J) iris and (K) sclera. Arrows represent pigment cells. Bars indicate 100 µm. Fontana-Masson staining.

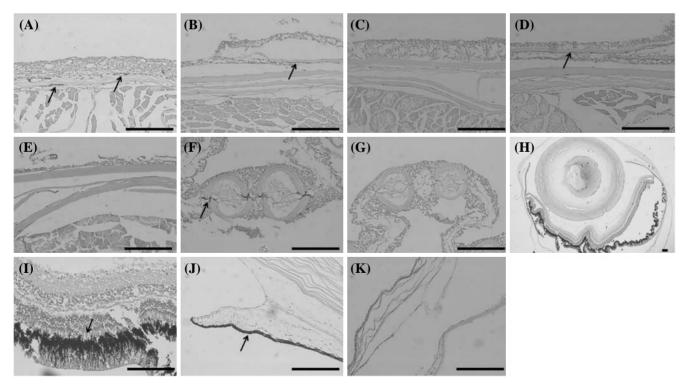


Fig. 5. Pigmentation of melanin cells on the eight regional skins in the albinic Korean slender gudgeon, *S. gracilis majimae*. (A) dorsal region, (B) lateral region, (C) ventral region, (D) upper caudal peduncle, (E) lower caudal peduncle, (F) dorsal fin, (G) anal fin, (H) the eyes, (I) retina, (J) iris and (K) sclera. Arrows represent pigment cells. Bars indicate 100 μm. Fontana-Masson staining.

2009) and *Silurus asotus* (Park *et al.*, 2009). The dermis consisted of dense connective tissue and numerous fibroblast cells. Scales rooted in the connective tissue of the dermis projected out of the skin. Pigment cells were abundant in the epidermis and dermis in the normal gudgeon but in the albinic one, it was much less or absent.

The eyes consisted of central lens, sensory retina, iris, cornea and sclera (Fig. 3H and 3I). The retina consisted of very elaborate structure of retinal pigment epithelium, visual cell layer, outer nucleus layer, inner nucleus layer and ganglion cell layer. These structures were similar to Korean bitterling *A. signifer* (Oh *et al.*, 2008). Pigmentation of melanin granule was active in the retinal pigment epithelium layer, the iris, and sometimes sclera region.

3. Distribution of pigment cell

In the normal gudgeon, the melanin pigment cells were widely distributed over the dorsal and the lateral region, particularly in the basement membrane of the epidermis and within connective tissue of dermis region (Fig. 4A and 4B), while they were not seen in the ventral region (Fig. 4C). Generally they were much more in the upper region of caudal peduncle (Fig. 4D) but seldom or vestigial in the lower caudal peduncle region (Fig. 4E). The dorsal and anal fin have well developed pigment cells (Fig. 4F and 4G), whereas it was rare or vestigial in the anal fin. In the eye, the pigment cells were much more over the retinal pigment epithelium region including the basement membrane of the retina and the inner region of the iris (Fig. 4I and 4J). There were pigment cell even in the sclera (Fig. 4K).

In the albinic gudgeon, the pigment cells were few or vestigial in the apical regions of the dorsal and lateral region (Fig. 5A and 5B), the upper caudal peduncle and the dorsal fin (Fig. 5D and 5F), but there did not existed over the ventral region, the lower caudal peduncle and the anal fin region (Fig. 5C, 5E and 5G). Although they were distributed by a small amount on the retinal pigment epithelium and iris of the eye (Fig, 5I and 5J), it was not seen at all in the basement membrane of the retina and outer region of the iris, unlike those of the normal one. They was vestigial or absent on the sclera (Fig. 5K).

Although the melanin pigment cells were less developed in albinic gudgeon, the melanin cells were mainly distributed in the upper part of the body, which it might be responsible for the light (Bolker and Hill, 2000).

REFERENCES

- Bolker, J.A. and C.R. Hill. 2000. Pigmentation development in hatchery-reared flat fishes. J. Fish Biol., 56: 1029-1052.
- Fukamachi. S., A. Shimada and A. Shima. 2001. Mutation

- in the gene encoding B, a novel transporter protein, reduce melanin content in medaka. Nature genetics, 28: 381-385
- Gronskov, K., J. Ek and K. Brondum-Nielsen. 2007. Oculocutaneous albinism. Orphanet Journal of Rare Diseases, 2: 1-8.
- Jegal, S.J., K.W. Ju, S.I. Kim, S.J. An, M.W. Lee, S.K. Kwak, K.Y. Hwang, T.J. Kim, H.D. Kim, Y.C. Shin, J.S. Kim, B.W. Min and W.S. Lim. 2006. Histotechnology, 4th ed. Koryeo medical publishing Co. 511pp. (in Korean)
- Kang, D.Y., H.C. Kim, J.H. Kim, K.G. Kim and J.I. Myeong. 2007. Effects of environment factors on the occurrence of pseudo-albinism in cultured flounder, *Paralichthys olivaceus*. J. Kor. Fish. Soc., 40: 234-242. (in Korean)
- Kim, I.S. and Y.J. Lee. 1984. Taxonomic review of the genus *Squalidus* (Cyprinidae, Pisces). Bull. Korean Fish. Soc., 17: 132-138. (in Korean)
- Kim, I.S., H. Yang, S.L. Lee and E.H. Lee. 2004. Karyotype analysis of the two species of the genus *Squalidus* (Pisces: Cyprinidae) in Korea. Korean J. Ichthyol., 16: 229-233. (in Korean)
- Kim, I.S., Y. Choi, C.R. Lee, Y.J. Lee, P.J. Kim and J.H. Kim. 2005. Illustrated book of Korean fishes. Kyo-Hak Publishing Co., Ltd., Seoul, 615pp. (in Korean)
- Koga, A. and H. Hori. 1997. Albinism due to transposable element insertion in fish. Pigment Cell Res., 10: 377-381.
- Lee, W.O., S.I. Jang, J.Y. Lee and S.J. Son. 1997. Comparison of morphological and chromosomal characteristics and cross breeding of the two types Korean mandarin fish, *Siniperca scherzeri*. Korean J. Ichthyol., 9: 228-234. (in Korean)
- Lin, J.Y. and D.E. Fisher. 2007. Melanocyte biology and skin pigmentation. Nature, 445: 843-850.
- Oh, M.K. and J.Y. Park. 2008. Seasonal change of the skin morphology of muddy loach, *Misgurnus anguillicaudatus* (Cobitidae) from Korea. Korean J. Ichthyol., 20: 90-96.
- Oh, M.K. and J.Y. Park. 2009. Seasonal variation of skin structure in a ricefield-dwelling mud loach *Misgurnus mizolepis* (Cobitidae) from Korea. Korean J. Ichthyol., 21: 87-92.
- Oh, M.K., J.Y. Park, M.J. You, E.J. Kang, S.G. Yang, E.O. Kim, Y.C. Jo, I.S. Park, C.H. Kim and I. Toshihiro. 2008. Histological Study of Oculocutaneous Albinism in the Korean Bitterling, *Acheilognathus signifier* (Osteichthyes; Cyprinidae). Korean J. Ichthyol., 20: 167-172.
- Park, J.Y. and I.S. Kim. 1999. Structure and histochemistry of skin of mud loach, *Misgurnus anguillicaudatus*

- (Pisces, Cobitidae), from Korea. Korean J. Ichthyol., 11: 109-116.
- Park, J.Y., I.S. Kim and S.Y. Kim. 2001. Morphology and histochemistry of the skin of the mud loach, *Misgurns mizolepis*, in relation to cutaneous respiration. Korean J. Biol. Sci., 5: 303-308.
- Park, J.Y., M.K. Oh, S.W. Yoon and W.O. Lee. 2009. Histological study of oculocutaneous albinism in Korean far eastern catfish *Silurus asotus*. Korean J. Ichthyol., 21: 100-105. (in Korean)
- Park, K.S., Y.P. Hong, S.S. Choi and K.K. An. 2005a. The spawning behavior of Korean slender gudgeon, *Squalidus gracilis majimae* (Cypriniformes: Cyprinidae). Korean J. Limnol., 38: 207-216. (in Korean)
- Park, K.S., Y.P. Hong, W.K. Moon, S.S. Choi and K.K. An. 2005b. The egg development of Korean slender gudgeon, *Squalidus gracilis majimae* (Cypriniformes: Cyprinidae). Korean J. Limnol., 38: 73-82. (in Korean)
- Presnell, J.K. and M.P. Schreibman. 1997. Humanson's animal tissue techniques. The Johns Hopkins Univ. Press, London, 572pp.
- Yoo, J.H., T. Takeuchi and K.S. Jeong. 2003. Effect of thyroid hormones and albinism during metamorphosis of flounder, *Paralichthys olivaceus* under low temperature stress. J. Korean Fish. Soc., 36: 65-67. (in Korean)

긴몰개 Squalidus gracilis majimae의 피부백색증에 대한 조직학적 연구

이용주・박종영1・오민기1

전주교육대학교 과학교육학과, 1전북대학교 자연과학대학 생물과학부 · 생물다양성연구소

요 약: 긴몰개 Squalidus gracilis majimae 백색증 개체가 자연에서 발견되어 조직학적 분석을 통해 정상개체 와의 차이를 비교하였다. 백색증 개체는 정상 개체에 비해 체색의 발현이 이루어지지 않았다. 등, 체측, 배, 미병부 상부, 미병부 하부, 등ㆍ뒷지느러미 및 안구조직에 대한 조직형태에서는 정상과 백색증 개체간 차이가 없었으나 멜라닌 색소의 분포 및 발현량에서 차이를 보였다. 정상 개체의 경우, 멜라닌 색소는 배쪽을 제외한 모든 부위에서 잘 발달한 모습을 볼 수 있었다. 그러나 백색증 개체의 경우, 멜라닌 색소는 눈과 몸통의 위쪽부위에서 매우 희미하게 관찰되었고, 아래쪽 부위(배, 미병부 하부, 뒷지느러미)에서는 전혀 발견되지 않았다. 이러한 멜라닌 색소의 분포는 빛과 연관이 있을 것으로 추측되었다.

찾아보기 낱말: 긴몰개, 백색증, 멜라닌 색소