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Regular Article

A Histological Study on the Visual Cell Layer of the Endemic Korean Species *Liobagrus mediadiposalis* (Pisces: Amblycipitidae)

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A study on the visual cell and eyeball of the endemic Korean species *Liobagrus mediadiposalis* was investigated by light and electron microscopes. The retina of a small and 2 mm-diameter round eye was thin, 151.0±4.0 μm and has two visual cells, a single cone and a rod cell. The single cone cells are short and thick, 18.0±0.9 μm in length and 5.1±0.7 μm (n=30) in diameter, while the rod cells are longer and thinner, 54.8±2.9 μm in length and 3.3±0.6 μm in diameter. The cone cells are seen an irregular and random mosaic pattern, and the rod cells are also randomly situated at between cone cells. As a rare phenomenon, such structure is one of characteristics reflecting the eye of a nocturnal and bottom-dwelling freshwater fish. The ultrastructure of visual cells was observed with scanning and transmission electron microscopy, both cone and rod cells are divided into an inner segment with numerous mitochondria and an outer segment with stacks of membrane discs.

Key Words: *Liobagrus mediadiposalis*, Retina, Visual cells, Nocturnal fish, Random mosaic pattern

INTRODUCTION

Water absorbs different amount of light by the depth of the water, which the deeper the depth is, the less light it absorbs. Also the optic properties of water also lead to different wavelengths of light such as long wavelengths (e.g., red, orange) being absorbed quickly or short one (blue, violet) being absorbed quite slowly (Engström, 1963; Marc & Sperling, 1976). In consideration of such water properties, fish vision is more important to feed on food items than other organs (an olfactory organ, a lateral line, and hairs on the jaw). Their retinas generally have both rod cells and cone cells (for scotopic and photopic vision).

Activity patterns of teleost in general are divided largely into diurnal or nocturnal fish although there are overlapping periods of the time during the activity, which is closely related to their feeding habits to seek prey regardless of the kind of food items (Nag & Bhattacharjee, 1993, 2002). Furthermore, the feeding action is variable upon the size and shape, and setting of the eyes with its retinal structures by species, based on aquatic ecosystems on whether they inhabit deep or swallow waters, or bottom or surface waters, or when they are activated, the day or the night.

Liobagrus mediadiposalis, a nocturnal and bottom-dwelling freshwater fish, is an endemic Korean catfish and preys on aquatic insects (Kim, 2013; Kim & Park, 2002). For the genus *Liobagrus*, except for ecological studies, the vision cells have been little known until now. Hence, this paper focused on studying the structure of the visual cells on the retina as part of understanding the relation between the vision and feeding habit surrounding its aquatic environment.

MATERIALS AND METHODS

During the non-spawning period, four males and two females were obtained from Gungnyu-myeon, Uiryeong-gun, Gyeongsangnam-do, the Nakdonggang River, Korea (35°26"N; 128°16"E). For general morphology, the eyes were extracted and observed by radial and tangential sections at right angles and parallel to the plane of the retina, respectively, after being anaesthetized with MS-222 (200 mg/L^{-1}) and fixed in 10% neutral buffered formalin. For light microscopy (LM), the fragmented samples were dehydrated through a standard ethanol series to 100%, cleared in xylene, and embedded in wax (Paraplast; Leica, Germany). Five-micrometer sections were deparaffinized and then stained with Harris hematoxylin and counter-stained with eosin (Gurr, 1956) for general histology. For photographs and evaluation of the eyes, Carl Zeiss vision was used (AX-10; Carl Zeiss, Germany). In addition, serial semi-thin sections (0.5~1.0 μm) were stained with toluidine blue and examined with the light microscope for gross morphology. For scanning electron microscopy (SEM), the fragments were prefixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.4. Post-fixation was performed in 1.0% osmium tetroxide in the same buffer. After dehydration in a graded alcohol series and drying to a critical point with liquid CO_2, the dried samples were coated with gold by ion sputtering and then examined with a SEM (S-450; Hitachi, Japan). For transmission electron microscopy (TEM), using the same method in fixation and dehydration as SEM, the specimens was embedded in Epon 812. The ultrathin sections of 60 to 70 nm were obtained in the double stained with 2% uranyl acetate and lead citrate on the grid. Observations were made at 7,000 to 30,000 magnification under 100 kV using a TEM (H-7650; Hitachi).

RESULTS

Morphology of the Eye

This fish, 85.6 to 93.7 mm standard length, has small round eyes, 2.1±0.2 mm, and are protruded slightly, and its horizontal and perpendicular eyes are nearly the same in size. The eye's diameter in percent of a small head and standard length is mean 11.9%±0.9% (n=10) and 2.3%±0.05% (n=10), respectively. The eyes are transparent and have no eyelids but are covered with fat (Fig. 1A).

Structure of the Visual Cells

In the radial sections by LM, the retina contains several layers: from the outermost layer to the layer closest to the vitreous body, a choroid layer, a retinal pigment epithelial layer, a visual cell layer, an outer nuclear layer, an outer plexiform layer, an inner nuclear layer, an inner plexiform layer, and a ganglion cell layer (Fig. 1B). In particular, the retinal pigment epithelial layer on the retina is situated just over the visual cell layer. The retinal pigment epithelium is the pigmented cell layer of pigment grains and melanin granules. The visual cell layer has two cells, a shorter single cone cell and a longer single rod cell (Fig. 1C). The single cone is 18.0±0.9 μm in length (n=30) and 5.1±0.7 μm (n=30) in diameter (n=30). Meanwhile, the rod is longer and thinner than the cone cell, 54.8±2.9 μm in length (n=30) and 3.3±0.6 μm (n=30) in diameter (n=30). Such visual cells are located between a retinal pigment epithelial layer and an outer nuclear layer. The cone cells are arranged in an irregular and random mosaic fashion, while the rods are also irregularly positioned at between the cone cells (Fig. 2A and B). With hematoxylin and eosin staining, the cone cells outer segment with hematoxylin and eosin staining are hematoxylinophilic, and with the semi-thin sections stained with toluidine blue, they are less positive. The rod cells outer segment being stained with hematoxylin, and are strong positive to toluidine blue. In SEM, both cone and rod cells are clearly divided into an inner segment and an outer segment (Fig. 1D). In TEM, the inner segment is packed with numerous membrane discs and the outer segment with

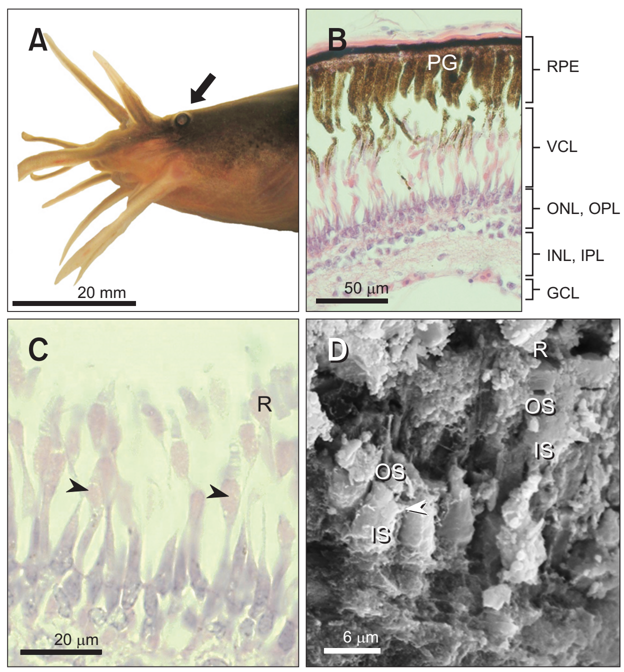

Fig. 1. External morphology of eyes and histological analysis of the visual cell layer in *Liobagrus mediadiposalis*. (A) The eyeball (arrow) of *L. mediadiposalis* is too small, about 2 mm. (B, C) Longitudinal sections of the visual cells in a Harris hematoxylin and counter-stained with eosin used by light microscope of *L. mediadiposalis*. (D) Scanning electron micrograph of the visual cells. PG, pigment granules; RPE, retina pigment epithelium; VCL, visual cell layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; R, rod cell; OS, outer segment; IS, inner segment; arrowheads, short single cone cells.

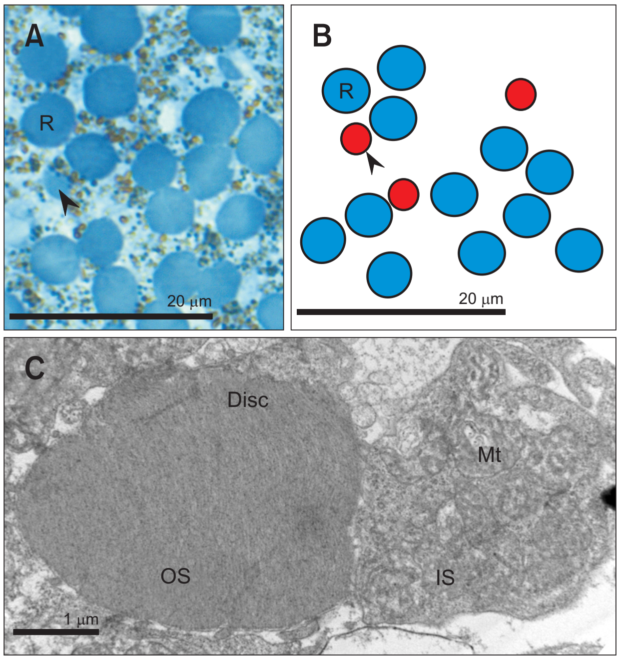

Fig. 2. Transverse sections of the visual cell layer and transmission electron micrograph of visual cell in *Liobagrus mediadiposalis*. (A) Transverse sections of the visual cell layer stained by toluidine blue. (B) Diagram based on the left micrograph. (C) Transmission electron micrograph of *L. mediadiposalis*. R, rod cell; Disc, membrane disc; OS, outer segment; Mt, mitochondria; IS, inner segment; arrowheads, short single cone cells.

numerous mitochondria (Fig. 2C).

DISCUSSION

A histological study by light, scanning and TEMs on the visual cell layer of the endemic Korean species *L. mediadiposalis* is revealed that this species has a general retina like other teleost. The thickness of the visual cell layer and its outer nuclear layer may be used as an indicator for the development of the visual cell in interspecies (Hagedorn et al., 1998; Lee & Lim, 2005; You & Park, 2008). For this fish, the thickness of the visual cell layer and the outer nuclear layer is 151.0±4.0 μm and 9.3±2.1 μm, while known as Korean benthic fishes similar to *L. mediadiposalis*'s habitat, *Iksookimia longicorpa* is 216.4±13.4 μm, 13.42±2.2 μm (Kim & Park, 2015), *Misgurnus angulicaudatus* 173.6±17.8 μm, 9.8±3.6 μm (Kim et al., 2015), and *Kichulchoia multifasciata* 191.2±13.7 μm, 10.4±1.9 μm (Kim & Park, 2017), respectively. This difference between *L. mediadiposalis* and three cobitid fishes may be related to their feeding activity (nocturnal vs. diurnal) although they all are gravel-covered bottom dwellers. The visual cells of *L. mediadiposalis* consist of a single cone and a rod cell. Among Korean fishes investigated on the visual cells, *Coreoperca herzi* consists of a double cone with the same size in two members, a short single cone and a rod, and *I. longicorpa* has a double cone with one long and one short member and a rod (Kim & Park, 2015; Kim et al., 2014). In *L. medidiaposalis*, the single cone cells are mean 18 μm in length and rod cells are mean 54.8±2.9 μm in length, while *M. angulicaudatus* 24.0 μm and 51.4 μm, *K. multifasciata* 21.6 μm and 53.3 μm, *I. longicorpa* 26.4 and 40.1 μm, respectively (Kim & Park, 2015, 2017; Kim et al., 2015). The cone cells of *L. mediadiposalis* are shorter and its rod cells are longer that those having similar ecological habitats. These features mean that the eyes of *L. mediadiposalis* are well adapted to the environments of a nocturnal fish. In general, as the optical properties of water cause to different wavelengths of light being absorbed to different degree, the fish need a various kind of cone cells to absorb such different wavelengths (Fernald, 1988; van der Meer, 1992). It is said that a single cone cell shows the highest absorption in the short wavelengths (green, blue), while double cone cells are the highest in the middle and long wavelength (red, orange) (Marc & Sperling, 1976; van der Meer, 1992). With such reports, it is possible that *L. mediadiposalis* enable feeding and survival in dim condition as a nocturnal fish. This fish with only a short single cone, however, is unlikely to distinguish color. With scanning and TEM, both cone and rod cells are divided into an inner segment with numerous mitochondria and an outer segment with stacks of membrane discs. In particular, the density of the mitochondria is different depending on the shape of the cone cells (Donatti & Fanta, 2007; Engström, 1963; Fernald, 1988). It is surmised that this fish's small cone cell contains less mitochondria than large cone cell, and that the ability to distinguish color is lower. The cone cells are seen an irregular and random mosaic pattern, and the rod cells are also randomly situated at between cone cells. By this study, such visual cells support strongly the ecological traits of a nocturnal and bottom-dwelling freshwater fish and the vision is more dependent on the rod cell than the cone cell being smaller (Fernald, 1982; Kunz, 1980; Nag & Bhattacharjee, 1993, 2002). In addition to the eye, long and well-developed hairs on the jaw may play a role in distinguishing between the food items and the objects in the night. Such hypothesis, however, will be further studied in the future.

CONCLUSIONS

The vision cells of an endemic Korean catfish, *L. mediadoposalis*, consist of a single cone cell and a rod cell, and the cone cells showed an irregular and random mosaic pattern. As a unique model in teleosts, this pattern may be closely related to its feeding activity at night as a nocturnal and benthic fish, and plays a role in understanding the relation between the vision and feeding habit surrounding its aquatic environment.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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