

Microscopic Organization of the Eye of Stoliczkae's Barb *Puntius stoliczkanus* (Day, 1871)

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ABSTRACT

The histology of the organization of the eye in the Stoliczkae's barb *Puntius stoliczkanus* was investigated using a light microscope. It was found that the eye in *P. stoliczkanus* comprised three layers—external, middle uveal and inner. The external layer consisted of the cornea and sclera. The cornea showed three layers: 1) corneal epithelium, 2) corneal stroma and 3) corneal endothelium, while the sclera exhibited a thick connective fiber and an external sheath of hyaline cartilage supporting the sclera. The middle uveal layer showed only the lens and choroid. The lens protruded into the aqueous chamber. The choroid was subdivided into two distinct layers: 1) a connective layer adjacent to the sclera and 2) the lamina vasculosa and choriocapillaris. The inner layer or retinal tunica was composed of 10 distinct layers: 1) pigment epithelium, 2) photoreceptor layer, 3) outer limiting membrane, 4) outer nuclear layer, 5) outer plexiform layer, 6) inner nuclear layer, 7) inner plexiform layer, 8) ganglion cell layer, 9) optic nerve layer and 10) inner limiting membrane, respectively, from the outside inward. These results provided data for other investigations in aquaculture, reproductive biology and histopathology.

Keywords: eye, histology, *Puntius stoliczkanus*, Tapee river, Thailand

INTRODUCTION

Generally, the organizational layers of the eyes of Teleostei of the fish class Actinopterygii can be distinguished into three different parts being the external, middle uveal and inner layers (Banks, 1993; Genten *et al.*, 2008). The external layer is covered with the eye-ball as a sclera-corneal layer, which is classified into two parts: the external corneal stroma and the internal sclera (Collin and Collin, 1995, 2001; Genten *et al.*, 2008). The middle uveal layer consists of the choroid, lens,

ciliary body and iris. The choroid is composed of three layers: 1) a connective layer, 2) the lamina vasculosa and 3) the lamina choriocapillaris, which is rich in small vessels and close to the retina. The optic nerve is commonly surrounded by the choroid rete mirabile, which is a well grown and intricate array of arterioles and capillaries. The function of this layer is maintained with a high pressure of oxygen that accommodates the retina's high oxygen demand (Wittenberg and Haedrich, 1974; Wittenberg and Wittenberg, 1974; Genten *et al.*, 2008). The inner layer or retinal layer in

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several fish species consists of 10 different layers beginning with the pigment epithelium through to the inner limiting membrane. This layer shows specialization as a duplex retina, with rods and cones as visual cells and is presented in the external limiting membrane (Genten *et al.*, 2008).

Among fish, eye histological studies have been conducted in several species including *Astronotus ocellatus*, *Barbus caudovittatus* (Genten *et al.*, 2008), *Bovichtus diacanthus*, *Cottoperca gobio* (Eastman and Lannoo, 2007), *Danio rerio* (Haug *et al.*, 2010), *Poecilia reticulata* (Wester and Canton, 1987; Wester *et al.*, 1990) and *Siganus javus* (Mansoori *et al.*, 2012; Sattari *et al.*, 2012) etc. Despite the interest in *Puntius stoliczkanus* as an ornamental fish in Thailand, the basic histology of the eye remains unknown. Hence, the purpose of this study was to clarify the detail of the organization of the eye of *P. stoliczkanus* to increase the histological knowledge of the eye of the Cyprinids found in Thailand.

MATERIALS AND METHODS

Twenty adult Stoliczkae's barb *Puntius stoliczkanus* were gathered during two periods, one in the rainy season (November, 2010) and the other in the dry season (April, 2011) with 10 individuals per sampling using a gill net in the Tapee river, Chawang district, Nakhon Sri Thammarat province, Thailand (8° 28.103' N, 99° 29.452' E). All fish were checked for normal health before being euthanized by the rapid cooling method (Wilson *et al.*, 2009). The head of each fish was decapitated and the eyes were immediately fixed in Davidson's fixative for 48 hr and then were processed using the standard histological technique. Sections (5 µm) were cut and stained with Harris's haematoxylin and eosin (H&E) (Humason, 1979). The structures and details of the eye were investigated using a light microscope following the method of Genten *et al.* (2008).

RESULTS AND DISCUSSION

The organization of the eye of *Puntius stoliczkanus* consisted of three distinct layers being the external, middle uveal and inner layers, similar to the structure in the higher vertebrates (Genten *et al.*, 2008). The external layer (the sclera-corneal layer) consisted of the cornea and sclera. This study identified three layers of the cornea of *P. stoliczkanus*: 1) the corneal epithelium which was presented as a simple squamous epithelium, 2) the corneal stroma or membranous stroma, which was the main constituent of the cornea and was contained with tightly connective bundles and 3) the corneal endothelium which was formed close to the surface of the cornea. The structure of the cornea related to the study by Collin and Collin (1995) who reported on pipefish *Corythoichthys paxtoni* and salamander fish *Lepidogalaxias salamandroides*. Sclera were exhibited in the wall of the posterior portion, which was made up of thick connective fibers. The external sheath of the hyaline cartilage supported the sclera (Figures 1 and 2).

In this study, the middle uveal layer revealed only the choroid which was subdivided into two distinct layers: 1) the connective layer adjacent to the sclerotic rings and 2) the lamina vasculosa and choriocapillaris with a network of capillaries, which was rich in small vessels and close to the retina. A horseshoe-shaped body in the choroidal layer of the eyes of bony fish is an aggregation of small, countercurrent blood vessels, known in many fish as the choroid rete of mirabile or choroid rete (Albers, 1806; Jones, 1838). It acts as a blood supply system that is made up of several thousand closely arrayed and parallel arterial and venous capillaries (Sattari *et al.*, 2012). According to Genten *et al.* (2008), the choroid rete is surrounded by the optic nerve and the function of the choroid rete is to meet the high oxygen demand from other parts of the eye (Figures 1 and 2).

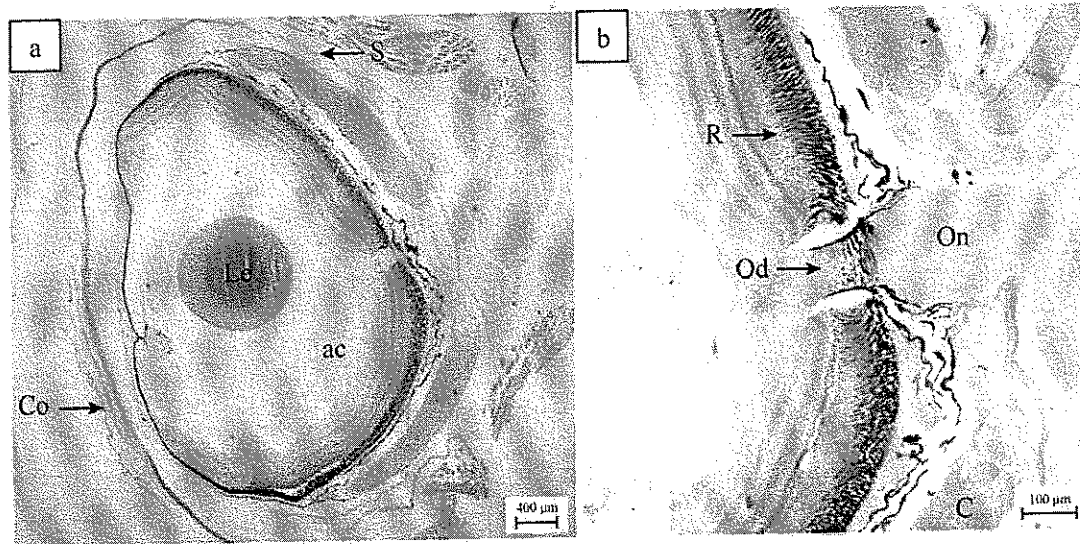


Figure 1 (a)–(b) Microscopic elements of the eyeball. (ac = Aqueous cavity; Co = Cornea; Le = Len; S = Sclera ; On = Optic nerve; Od = Optic disc or optic papilla; R = Retina.)

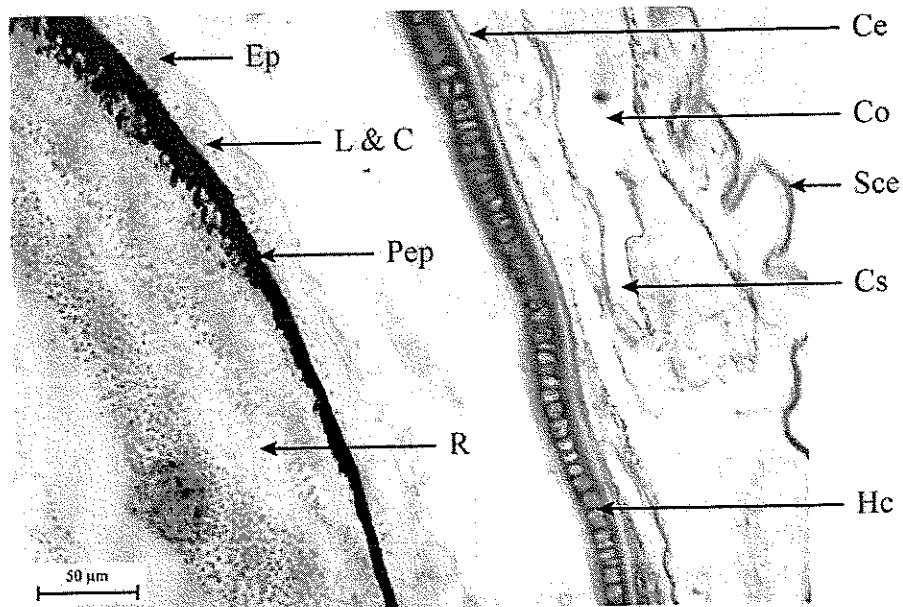


Figure 2 Section through the cornea (lateral side of the eye). In this micrograph, the layers of the cornea can be seen: 1) squamous corneal epithelium, 2) corneal stroma and 3) thin corneal endothelium. (Ce = Corneal endothelium; Co = Cornea; CS = Corneal stroma; Ep = Epithelium; Hc = hyaline cartilage; L & C = Lamina vasculosa and choriocapillaris; Pep = Pigment epithelium; R = Retina; Sce = Squamous corneal epithelium.)

The inner layer or retinal tunica is comprised of ten distinct layers (Figure 3): 1) the pigment epithelium is brown and contains melanin and is closely apposed to the choroid; 2) the photoreceptor layer that might be associated with rod and cone processes. In various fish, the photoreceptor consists of rod and cone cells, where the rod cell is longer than the cone cell and ellipsoid while the cone cell is conical and a rather bulbous ellipsoid. The rods function is to detect dim light conditions while the cones are very sensitized to the red, green or blue regions of the visible spectrum. However, in some fish species, this layer is composed of only rods such as deep-sea fish (Genten *et al.*, 2008) where 3) the outer limiting membrane is not clearly visible; 4) the outer nuclear layer is clearly visible, which contains the nuclei of the photoreceptors cells; 5) the outer plexiform layer; 6) the inner nuclear layer consists of bipolar cells, which are connected

to photoreceptors; 7) the inner plexiform layer; 8) the ganglion cell layer, where the nuclei pick up the stimuli from the bipolar elements and send them to the fibers; 9) layer of ganglion cell axons forming the optic nerve or the nerve fiber layer along the optic nerve to the brain; and 10) the inner limiting membrane. The retina of vertebrates is organized in superimposed layers, formed by different cells and contains five major cell types, being the photoreceptors (rods and cones) and bipolar, horizontal, amacrine and ganglion cells. Ganglion cells transmit a message to the brain in the form of action potentials, which are influenced by amacrine which the ganglion cells receive from the bipolar cells (Méndez-Vilas and Díaz, 2010). Some fish species, such as the Molino cavefish, can be distinguished by showing a slightly lesser degree of reduction in eye-size and pigmentation (Wilkins and Strecher, 2003).

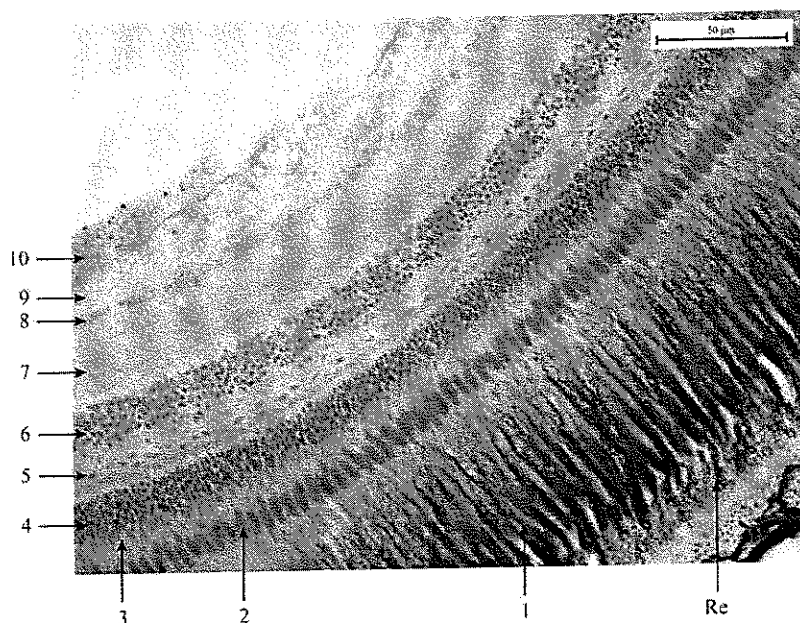


Figure 3 Retinal layers backed by a heavily vascularized choroid: 1) pigment epithelium mostly devoid of melanin, 2) photoreceptor layer (rod and cone processes), 3) outer limiting membrane, 4) outer nuclear layer consisting of the nuclei of the photoreceptors, 5) outer plexiform layer, 6) inner nuclear layer, 7) inner plexiform layer, 8) ganglion cell layer, 9) nerve fiber layer and 10) the internal limiting membrane. (Re = Retinal pigment epithelium).

CONCLUSION

The present study on the eye histology of *Puntius stoliczkanus* described the foundation of the histological organization, which conformed to other fish species in tissue appearance. This finding provides useful information for other studies in aquaculture, reproductive biology and histopathology.

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