Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60366-9 

# Histological study of middle layer of rabbit fish eye (*siganus javus*)

Amir Sattari[1*], Marziyeh Asli[2], Foroogh sadat Mansoori[3], Reza Kheirandish[4], Hossein Yavari[5]

[1]*Department of Food Hygiene and Quality Control, Division of Aquatic Animal Health, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran*
[2]*Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran*
[3]*Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran*
[4]*Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran*
[5]*Iranian Veterinary Organization, North Kharasan Veterinasy Head Office, Bojnoord, Iran*

ARTICLE INFO

*Article history:*
Received 5 June 2012
Received in revised from 5 July 2012
Accepted 7 August 2012
Available online 28 August 2012

*Keywords:*
*Siganus javus*
Histology
Eye
Choroid
Iris

ABSTRACT

**Objectives:** This study investigates the histology of the middle layer of the eye in rabbit fish Siganus javus. **Methods:** The eyes of twelve healthy fish were enucleated and histologic sections of 6μ were prepared. The sections were stained with Hematoxyline & Eosine (H&E) and Masson trichrom then were observed using light microscopy. **Results:** The middle or vascular layer consisted of the choroid and iris. The result revealed that the choroid coat was subdivided into five laminae: 1. Suprachoroid layer 2. Substina propria included choroidal gland or retemirabile, melanoid layers and connective tissue 3. Fibrouse layer 4. Choriocapillary layer 5. Separator layer. The extension of the choroid coat into the anterior compartment made the iris which mainly composed of connective tissue, blood vessels, melanocyte and a smooth muscle at the posterior part of the iris, adjacent to the pupil. The choroid coat did not continue rostrally adjacent to the cornea. Suspensory apparatus of the lens of the Siganus javus eye consisted of a dorsal suspensory ligament and a ventral retractor muscle. **Conclusions:** The diversity and deviation in the detail of microscopic structures in the eye of teleosts is approved through this microscopic study in middle layer of rabbit fish eye however the five differentiated parts of choroid were distinguished as in many telelost species.

## 1. Introduction

The wall of the eyeball is composed of three layers: the outer layer or fibrouse tunic, the middle or vascular layer and the inner or nervous layer. The vascular layer consists of the choroid and iris[1].

In many fishes there is a large discrete organ lying within the eyeball behind the retina in choroid layer which is named choroid rete mirabile. It acts as a blood supply system made up of several thousand closely arrayed and parallel arterial and venous capillaries[2]. The choroid rete, in conjunction with the pigment cell epithelium, maintains a large pressure of oxygen at the retina[3]. The iris is the continuation of the choroid into the anterior compartment. The free margin of the iris defines the pupil. It mainly composed of connective tissue, blood vessels, melanocyte and a smooth muscle at the posterior part[1]. Teleosts have a variety of muscles within their irises[4]. It appears many lower vertebrates posses a sphincter muscle in the iris for constriction of the pupil and pupil dilation results from relatively slow passive processes[5,6]. In mammals the ciliary process is the most rostral extension of the ciliary body at the base of the iris. Zonular fibers pass from it to suspend the lens [1] but the eye of teleost do not have ciliary bodies and the lens is suspended by a suspensory ligament on the dorsal side and a tendon of the muscle on the ventral side [7-9]. Accurate recognition of fish physiologic conditions and normal structures are proper ways to determine fish-culture needs. Information of morphological, macroscopic and microscopic features of fish tissues can help to develop cellular biology and provide pathologic evaluation of diseases and lesions. Histological research plays often an important role to gain these kinds of results.

Two species of rabbit fish recognized in southern sea of Iran living in shallow coastal waters with aquatic plants are *Siganus Sutore* and *Siganus javus* which are economically valuable teleosts for human consumption and marine culture feeding.

Although fish histological studies in numerous species were performed the histology of the *Siganus javus* eye

*Corresponding author: Amir Sattari, Department of Food Hygiene and Quality Control, Division of Aquatic Animal Health, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
Tel: 00989131997581
Fax: 00983413222047
E-mail: amirsatari@uk.ac.ir

has unlikely investigated. In the current study, the middle layer of the eye in *Siganus javus* was investigated using Hematoxyline and Eosin (H&E) and Masson trichrom staining methods.

## 2. Materials and methods

12 male rabbit fish of 365 ± 100 g bodyweight, where chosen from marine fish propagation and growing center of Booshehr province (south of Iran). All of them were healthy and in good conditions, without any external deformities and lesions. The fish were decapitated then their heads were immersed in 10% formalin solution and kept for 7 days after which their eyes were enucleated. Sections of 6 micron thickness were prepared from eye through routine histological techniques (Slee® microtome; Germany and tissue processor Didban sabz DS2080/H; Iran) and were stained with standard H&E and Masson trichrom. Histological study was done using light microscopy and photographs were taken for detailed illustration of the results.

## 3. Results

The choroid coat was subdivided into these laminae: 1. suprachoroid layer 2. substina propria 3. Fibrouse layer 4. Choriocapillary layer 5. Separator layer (Figure 1, 2, 3).

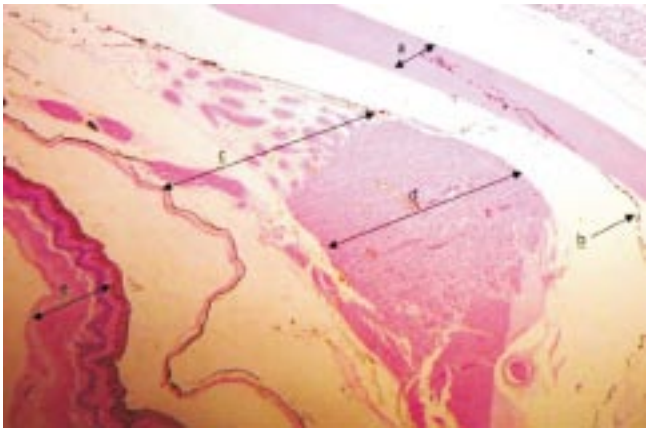

**Figure 1.** Middle layer position into the outer and inner one.
a: scleral ossicle; b: suprachroid layer; c: choroid coat; d: choroidal gland (rete mirabile); e: retina (×40) H&E.

### 3.1. Suprachoroid layer

suprachoroid layer was a vascular and most prepheral lamina that separated choroid and sclera and made of connective tissue, fibers and pigment cell (Figure 1, 2, 3).

### 3.2. Substina propria

Substina propria included connective tissue, pigmented layers and blood vessels. Expansion of the blood vessels made two parts crescent shaped glands or rete mirabile situated in dorsal and ventral of the globe. Substina propria were surrounded by two pigmented layers, one next to the suprachoroid layer and the other beside the fibrouse lamina. (Figure 1, 2, 3).

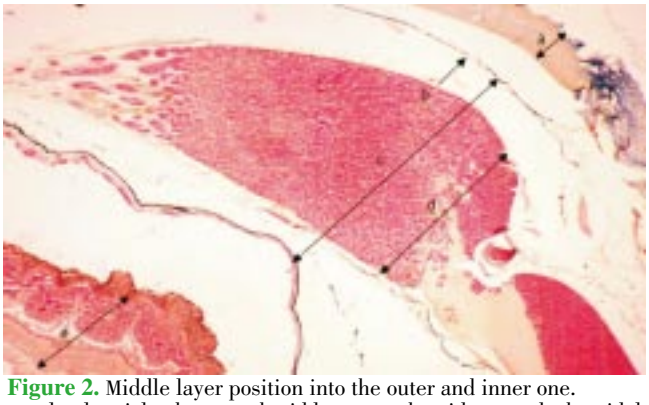

**Figure 2.** Middle layer position into the outer and inner one.
a: scleral ossicle; b: suprachroid layer; c: choroid coat; d: choroidal gland (rete mirabile) e: retina (×40) masson trichrom.

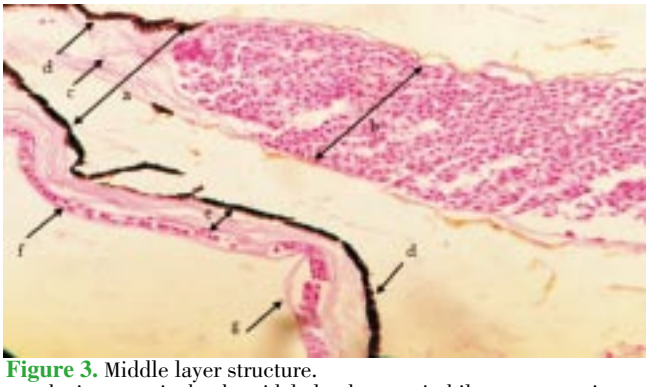

**Figure 3.** Middle layer structure.
a: substina propria; b: choroidal gland ( retemirabile); c: connective tissue; d: pigmented layer; e: fibrouse layer; f: choriocapillary layer; g: separator layers (×400) H&E.

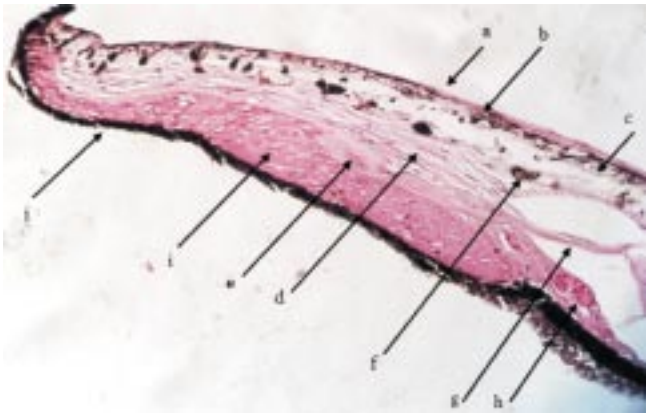

**Figure 4.** Iris structure.
a: cranial smooth border; b: melanocyte; c: anterior loose connective tissue; d: loose connective tissue of iridial stroma; e: blood vessels; f: melanocyte; g: posterior dense connective tissue; h: blood vessels; i: smooth muscle; j: presence of melanin pigment in both pigmented and nonpigmented epithelium adjacent to the pupil (×400) H&E.

### 3.3. Fibrouse layer

The fibrouse layer between the choriocapillary and pigmented layers made of dens connective tissue (Figure 3).

### 3.4. Choriocapillary and separator layers

The choriocapillary layer was rich in capillaries and situated next to the complex of connective tissue as a separator layer which was the interface between the choriocapillary layer and the pigmented epithelium of the retina (Figure 3).

The extension of the choroid coat into the anterior compartment made the iris. The cranial smooth border of the iris was covered by a discontinuous combination of the pigment cell and connective tissue fibers. The spongy iridial stroma in the middle of the iris comprised the reticular fibers of loose connective tissue, blood vessels and pigment cells. Posterior part of the iris composed of dense connective tissue, blood vessels, pigment cells and a smooth muscle. The coudal border of the iris was covered by a non pigmented rough epithelium backed by a pigmented layer. These two layers continued next to the pupil where there was an increasing number of melanin to be found in the non pigmented layer until the two layers were hard to distinguish (Figure 4, 5).

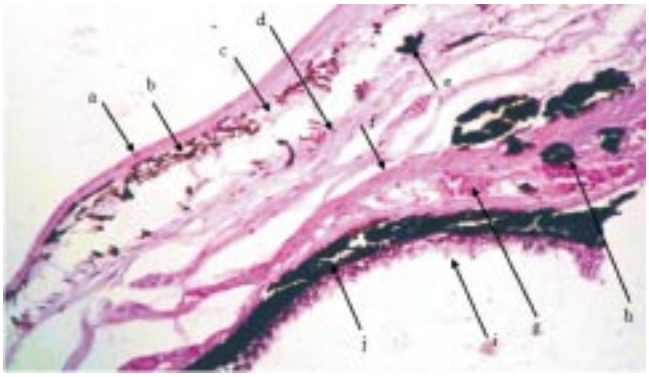


**Figure 5.** Microscopic elements of the iris.
a: cranial smooth border; b: melanocyte; c: anterior loose connective tissue; d: loose connective tissue of iridial stroma; e: melanocyte f: posterior dense connective tissue; g: blood vessels; h: melanocyte; i: nonpigmented epithelium of coudal border; j: pigmented epithelium of coudal border (×400) H&E.

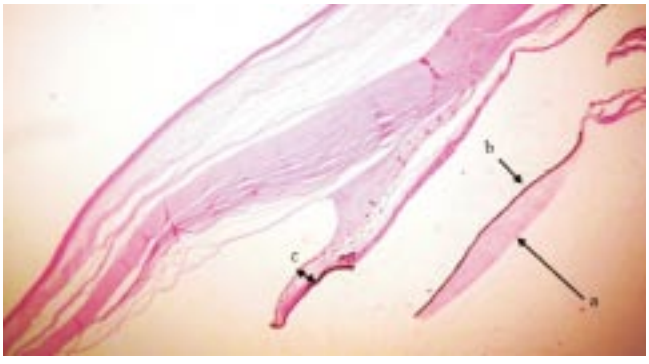


**Figure 6.** Suspensory apparatus of the lens and the position into the iris.
a: suspensory ligament; b: anterior pigmented layer of suspensory ligament; c: iris (×40) H&E.

Suspensory apparatus of the lens of the *Siganus javus* eye consisted of a dorsal suspensory ligament which made up of dense regular connective tissue covered by a pigmented layer cranially and a ventral muscle both situated beneath the iris (Figure 6).

The middle layer or vascular tunic of the *Siganus javus* eye consisted of the choroid coat and the iris. The choroid coat as a highly vascularized region between the sclera and the retina did not continue rostrally adjacent to the cornea (Figure 7, 8).

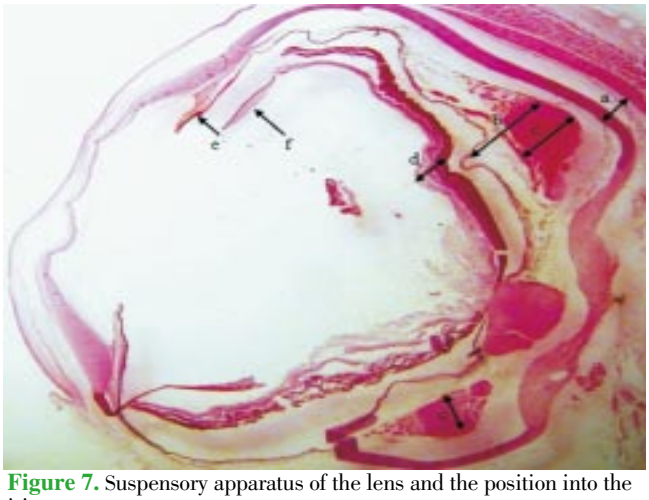


**Figure 7.** Suspensory apparatus of the lens and the position into the iris.
a: retractor muscle; b: iris; c: choroidal gland (retemirabile); d: retina; e: iris; f: suspensory ligament (×20) H&E.

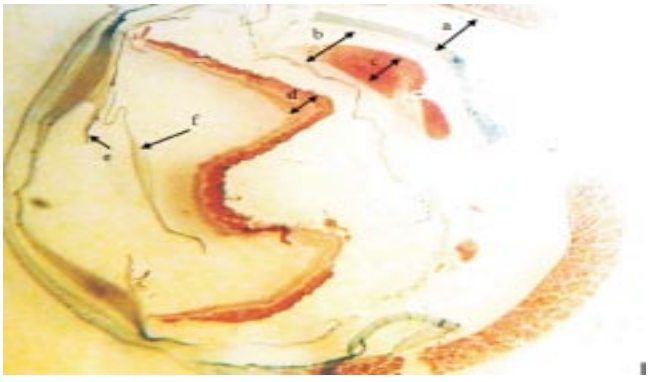


**Figure 8.** Sajital section of the eye.
a: sclera; b: substina propria; c: choroidal gland (retemirabile); d: retina; e: iris; f: suspensory ligament (×20) H&E.

## 4. Discussion

In this study the choroidal vessels were bilaterally well developed at the upper, downer and external side of the vascular choroid making rete mirabile. A horseshoe shaped body in the choroidal layer of the eye in most bony fish is an aggregation of small blood vessels and the suggested name for the choroidal vascular structure is choroid rete mirabile[10,11]. Distribution of the choroid rete among fishes was investigated and choroid rete in both shallow and deep living fishes was found[2]. For instance *Amia calva* as the only non teleost fish had rete mirabile in the eye and it is confirmed the choroid rete of the fast swimming sight

dependent predaceous fish is greatly developed[3]. Barnett explained the most detailed description of the choroidal circulation and a smaller structure, the lentiform body, also supplying blood to the choriocapillaries[12]. Some bony fishes possess falci form process in choroid layer which receives blood from the lenti form body[13] but these two parts were not observed by light microscopy in the eye of *Siganus javus*. The other part named tapetum lucidum is fibrouse or cell layer of the choroid peripheral to the choriocapillary layer, often described as a light reflective surface[1]. In several teleosts using conventional light and fluorescence microscopy the eye shine phenomenon approved to be related to the presence of a reflecting surface in the eye; tapetum lucidum[14]. Two types of tapetum, retinal and choroidal exist in the eye that choroidal one situated in the vitread region of the choroid and the main reflecting material in this layer is guanine also in a number of profound living fishes[5,6].

The only component of the layer with the same situation in the *Siganus javus* eye observed by ligh microscopy was connective tissue fibers so we name it as a fibrous layer, between the choroid coat and choriocapillary layer.

The separator layer of connective tissue which was in correlation with the choriocapillary layer of the rabbit fish eye, exist in many fishes and includes three laminae among teleosts[13].

Presence of a smooth muscle in the rabbit fish iris can introduce the ability of pupil rsponse to the light stimulation and pupilary constriction. Certain groups of bony fish display significant pupil mobility. The eel, for example, was the first teleost shown to have extensive pupil constriction and some armoured catfish, such as Plecostomus show extensive pupillary change[15,8].

The observed suspensory apparatus of the lens in the eye of *Siganus javus* by light microscopy is in accordance to the classic description[8], but additional evaluation in some species of teleosts revealed that the lens suspension is more complex than previously described as a complex system of ligaments and a muscle keep the crystalline lens in place in the eye of bony fishes[16].

The diversity and deviation in the detail of microscopic structures in the eye of teleosts is approved through this microscopic study in middle layer of rabbit fish eye however the five differentiated parts of choroid were distinguished as in many telelost species.

## Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgments

This project was funded by the veterinary faculty of Shahid Bahonar University of Kerman. The authors are thankful to Mr. Hasanzadeh gave much histological assistance and was responsible for the photographs.

## References

[1] Banks WJ. *Applied veterinary histology*. 3rd ed. Mosby year book; 1993, p. 469-470.

[2] Wittenberg JB, Haedrich RL. The choroid rete mirabile of the fish eye. II. Distribution and relation to the pseudobranch and to the swimbladder rete mirabile. *Biol Bull* 1974, **146**: 137-156.

[3] Wittenberg JB, Wittenberg BA. The choroid rete mirabile of the fish eye. I. Oxygen secretion and structure: comparison with the swimbladder rete mirabile. *Biol Bull* 1974; **146**: 116-136.

[4] Grynfeltt E. Les muscles de l'iris chez les teleostiens. *Bibl Anat* 1909; **20**: 265-332.

[5] Douglas RH, Partridge JC, Marshal NJ. The eyes of deep-sea fish I: Lens pigmentation, tapeta and visual pigments. *Prog Retin Eye Res* 1998; **17**: 597-636.

[6] Douglas RH, Harper RD, Case JF. The pupil response of a teleost fish, Porichthyes notatus: description and comparison to other species. *Vision Res* 1998; **38**: 2697-2710.

[7] Copeland DE. The anatomy and fine structure of the eye in fish. VI ciliary type tissue in nine species of teleosts. *Biol Bull* 1982; **163**: 131-143.

[8] Walls GL. *Higher fishes: The vertebrate eye and its adaptive radiation*. New York: McGraw-Hill; 1942, p. 577- 583.

[9] Duke-Elder S. *The eye in evolution, system of ophthalmology*. London: Klimpton; 1958, p. 293-302.

[10] Albers JAA. Uber das Auge des Kabeljau Gadus morhua und die Schwimmblase der Seeschwalbe, Trigla hirundo. *Gottingen gelehrte Anseiger* 1806; **2**: 681 -682.

[11] Jones TW. On the so-called choroid gland or choroid muscle of the fish's eye. *London Med Gas* 1838; **21**: 650-652.

[12] Barnett CH. The structure and function of the choroidal gland of teleostean fish. *J Anat* 1951; **85**: 113-119.

[13] Kunz YW. Development of the eye. In: *Developmental biology of teleost fishes*. 1st ed. Springer; 2004, p. 303-330.

[14] Somiya H. Fishes with eye shine: functional morphology of guanine type tapetum lucidum. *Mar Ecol Prog Ser* 1980; **2**: 9-26.

[15] Studnitz G von. Beträge zur adaptation der teleosteer. Studien zur vergleichenden physiologie der iris II. *Z Vg Physiol* 1932; **18**: 307-338.

[16] Khorramshahi O, Schartau JM, Kroger RHH. A complex system of ligaments and a muscle keep the crystalline lens in place in the eye of bony fishes. *Vision Res* 2008; **48**: 1503-1508.