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COMPARATIVE MORPHOLOGICAL STUDIES ON THE VASCULAR TUNIC OF THE EYEBALL OF TWO SPECIES OF FISHES: *OREOCHROMIS NILOTICUS AND MUGIL CEPHALUS*

(With 13 Figures)

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دراسات مورفولوجية مقارنة على الغلالة الوعائية لمقله العين لنوعين من الأسماك: البلطى النيلى والبورى

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تتكون الغلالة الوعائية لمقله العين لسمك البلطى والبورى من مشيمية خلفية وقزحية أمامية. تتكون المشيمية بشكل أساسى من طبقة وعائية محددة من الخارج بطبقة الأرجنته، وتتمثل بطبقة بنية فاتحة ذات سمك متماثل تقريبا تتكون من خلايا مغزلية أو عصوية. على عكس الحال فى البورى فان طبقة الأرجنته فى البلطى تكون مرتبطة بالميلانين. تتكون الطبقة الوعائية فى الجزء الامامى من المشيمه من صف واحد من الأوعية الدموية رقيقة الجدار والتى تتمدد لتكون شبكة وعائية تعرف بغدة المشيمية فى الجزء الخلفى منها وحيث يعتقد أن لها وظيفة حركية دموية. تمثل القزحية الإمتداد الأمامى للمشيمية حيث تشبهها فى التركيب الأساسى. وتغطى القزحية عند قاعدتها من الأمام بطبقة واحدة من النسيج الطلائى المفلطح ومن الخلف بنسيج طلائى ثنائى الطبقات، خارجى بصبغة الميلانين وداخلى غير مصبوغ، على أن الجزء الأمامى من القزحية يغطى من الخلف فقط بالطبقة المصبوغة بالميلانين. كما أظهرت الدراسة أن قزحية البورى تحتوى على ألياف عضلية ملساء قرب حافة البؤبؤ والتى قد تلائم حياة البرك فى هذا النوع من الأسماك.

SUMMARY

The vascular tunic of the eyeball of both *Oreochromis niloticus* and *Mugil cephalus* consists of a posterior choroid and an anterior iris. The choroid consists mainly of a vascular layer bordered externally by the choroidal argenta. The latter is represented by a light brown, more or less uniformly thick layer of fusiform or rod shaped cells separating the choroid from the sclera. Unlike that of *Mugil cephalus*, the argenta of

Oreochromis niloticus is pigmented. The vascular layer of the choroid appears in the form of one layer of thin walled vessels that widen posteriorly forming a vascular plexus; the choroid gland or Rete mirabile, which is suggested to have a haemodynamic function. The iris is the anterior continuation of the choroid with which it is similar in general structure. The base of the iris is covered anteriorly by a layer of flat epithelium and posteriorly by a bilayered epithelium. The inner one of the latter is non-pigmented, but the outer layer is pigmented. Near the pupillary margin, the iris of *Mugil cephalus* shows a thin layer of smooth muscle cells that may be an accommodation for the brackish water habitat of this species of fishes.

Key words: *Eye, fish, vascular coat*

INTRODUCTION

The eye is the highly specialized sense organ in the body. The eyeball (*Bulbus oculi*)consists of three tunics: (1) The outer fibrous tunic (*Tunica fibrosa bulbi*), (2) The middle vascular tunic (*Tunica vasculosa bulbi*), and (3) the inner nervous tunic (*Tunica interna bulbi*) or retina, with (a) an optic portion containing the sensory receptors and (b) a blind portion that is epithelial in nature and covers the posterior surface of the ciliary body and iris (Dellmann, 1976). The eye of fishes lacks ciliary body (Lanzing and Wright, 1981).

The structure of the eyeball of many species of fishes have been extensively studied by several authors (Breakevelt *et al.*, 1998a & 1998b; Bozzano *et al.*, 2001; Haacke *et al.*, 2001; Rodriguez and Gisbert, 2001). However researches on the vascular coat of the fishes is meager (Haacke *et al.*, 2001).

The aim of this work is to clarify the structural characteristics of the vascular tunic of the eyeball in two species of fishes with different habitats: *Oreochromis niloticus* and *Mugil cephalus*.

MATERIALS and METHODS

The present work was carried out on 5 eyeballs from each of the *Oreochromis niloticus and Mugil cephalus* obtained from mature and clinically healthy fishes.

The eyeballs were carefully extracted, dissected from the surrounding periorbital fat and extraocular muscles. For light microscopy, three eyeballs from each studied fishes were obtained. For

paraplast embedding, small pieces were taken from different parts of the vascular coat, fixed in Bouin's solutions for 24 hours. After proper fixation, the specimens were dehydrated in graded ethanol, cleared in methyl benzoate, embedded in paraplast and sectioned at 3-5 μm thick. The prepared sections were stained with haematoxylin and eosin for general histological description and trichrome for detection of muscles (Drury, Wallington and Cameron, 1967). For scanning electron microscopy two eyeballs from each studied fishes were used. Small pieces of the eyeball with the two parts of the vascular tunic namely; choroid and iris were fixed in a mixture of paraformaldehyde solution (2.5%) and glutaraldehyde solution (2.5%) in phosphate buffer for 24 hours. The specimens were then washed in 0.1M phosphate buffer (7.3 pH), dehydrated in ascending graded ethanol, critical point - dried in liquid carbon dioxide, then coated with gold palladius in sputtering device. The specimens were then examined and photographed using JSM-5400 LV Scanning electron microscope operated at 20 KV in the EM center of Assiut University. The choroid was scanned on its cut surface to visualize its layers. The iris was scanned on their posterior surface.

RESULTS

The vascular tunic of the eyeball of both examined fish species consists of a choroid posteriorly and an iris anteriorly. The vascular tunic in both species lacks ciliary body. For convenience, the two parts of the vascular tunic will be described at scanning microscopical level then by light microscopy.

Scanning electron microscopy:

The choroid of both examined fish species (*Oreochromis niloticus* and *Mugil cephalus*) as demonstrated in cut sections, appears consisting mainly of a network of thin walled vessels that form one layer in the anterior part, but they break down into a multilayered plexus posteriorly. This vascular network is bordered externally by the argenta which is demonstrated in the form of a more or less uniform compact layer on the inner aspect of the sclera (Figs. 1,2).

The choroid continues anteriorly as the iris, from which it is separated by an Ora serrata. The latter is demonstrated at the junction of the pigmented retinal epithelium and the posterior iridal epithelium. The retinal pigmented epithelium shows relatively long processes that shorten strongly at the Ora serrata then relatively elongate again in the iridal zone. *Oreochromis niloticus* has a very clear *Ora serrata* in comparison to the weakly developed one in *Mugil cephalus* (Figs. 3, 4).

In *Oreochromis niloticus*, the posterior iridal epithelium displays a relatively long processes with variably shaped openings in between. However the posterior iridal epithelium of *Mugil cephalus* is studded by numerous microvilli in addition to irregular low ridges and few small variably shaped openings in between (Figs. 5,6).

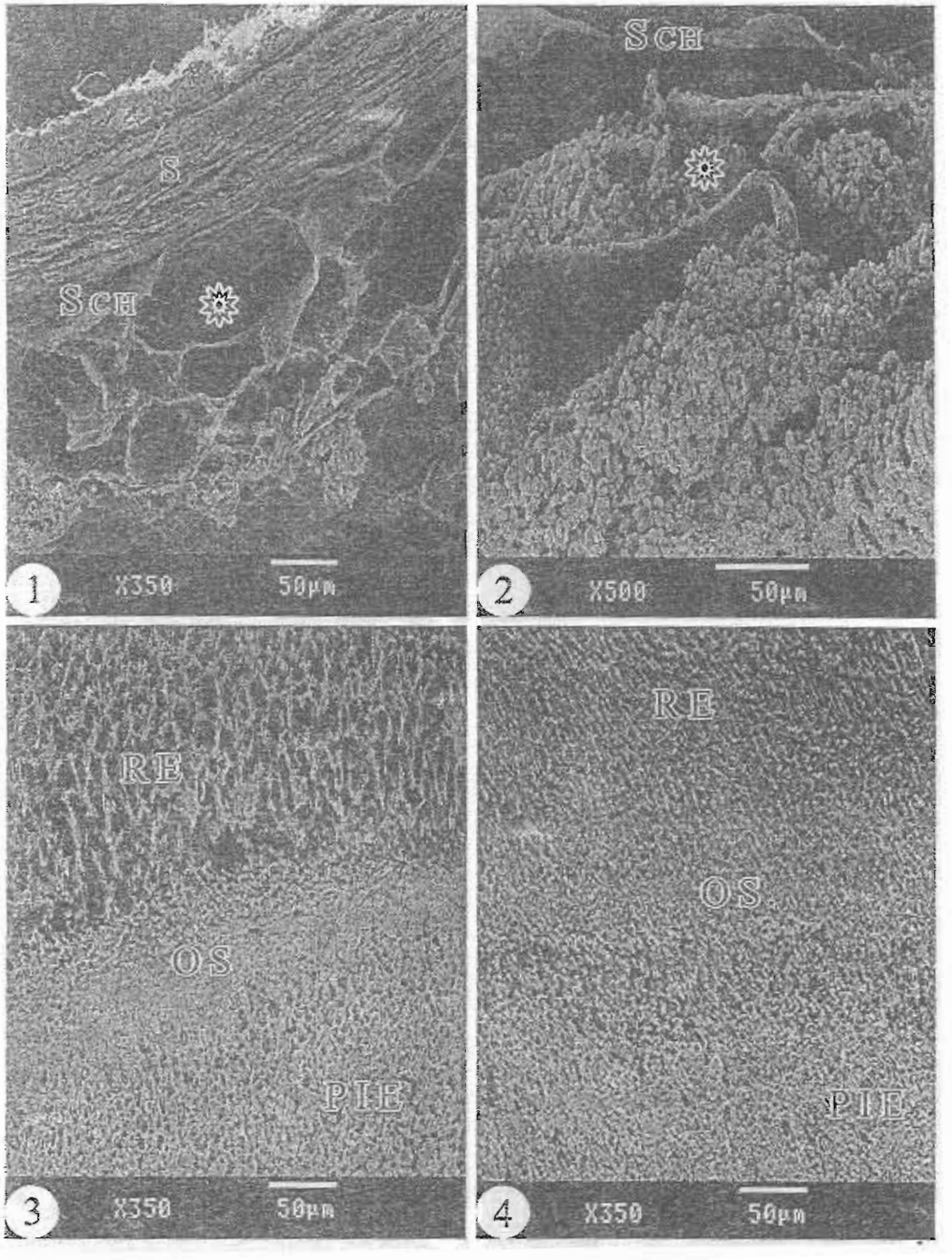
The iridocorneal angle is clearly demonstrated in both examined species in the form of variably sized and shaped openings. In cut section it appears in the form of irregular network of connective trabeculae hosting a plexus of channels (Figs. 7,8).

Light microscopy:

Histologically, the choroid consists mainly of a vascular network (formed mainly of thin walled veins) arranged in a thin single layer anteriorly, but posteriorly it widens several times forming an interconnected vascular plexus. The above mentioned plexus appears hosted in thin intervascular spaces filled with fine connective tissue stroma that shows melanin pigmentation only in *Oreochromis niloticus*. The argenta which appears in the form of a light brown relatively thick multicellular layer separates the choroidal vascular plexus from the sclera. The argental cells are either fusiform or rod shaped cells arranged in several well packed layers. Abundant melanin pigmentation is visualized only in the argenta of *Oreochromis niloticus* (Figs. 9,10).

The iris represents the anterior continuation of the choroid. Structurally, it resembles that of the choroid where it consists of an inner vascular layer bordered externally by the argenta of the iris. The vascular layer forms one continuous layer of thin walled veins extending almost to the pupillary border of the iris. The argenta (relatively thicker in *Mugil cephalus*) represents the direct anterior extension of that of the choroid. Unlike the case in *Mugil cephalus*, the argenta of *Oreochromis niloticus* showed intensive pigmentation. The iris of *Mugil cephalus* has a thin layer of smooth muscle fibers near its pupillary margin (Figs. 11, 12).

The iris is covered anteriorly by a flat epithelial layer that represents the continuation of the corneal endothelium. The thin subendothelial connective tissue layer shows melanin pigmentation only in *Oreochromis niloticus*. Near the choroid, the posterior iridal epithelium consists of two layers, an outer pigmented (continuation of the retinal pigmented epithelium) and an inner non pigmented (continuation of the sensory retinal layers) that fades out toward the pupillary margin (Figs. 13).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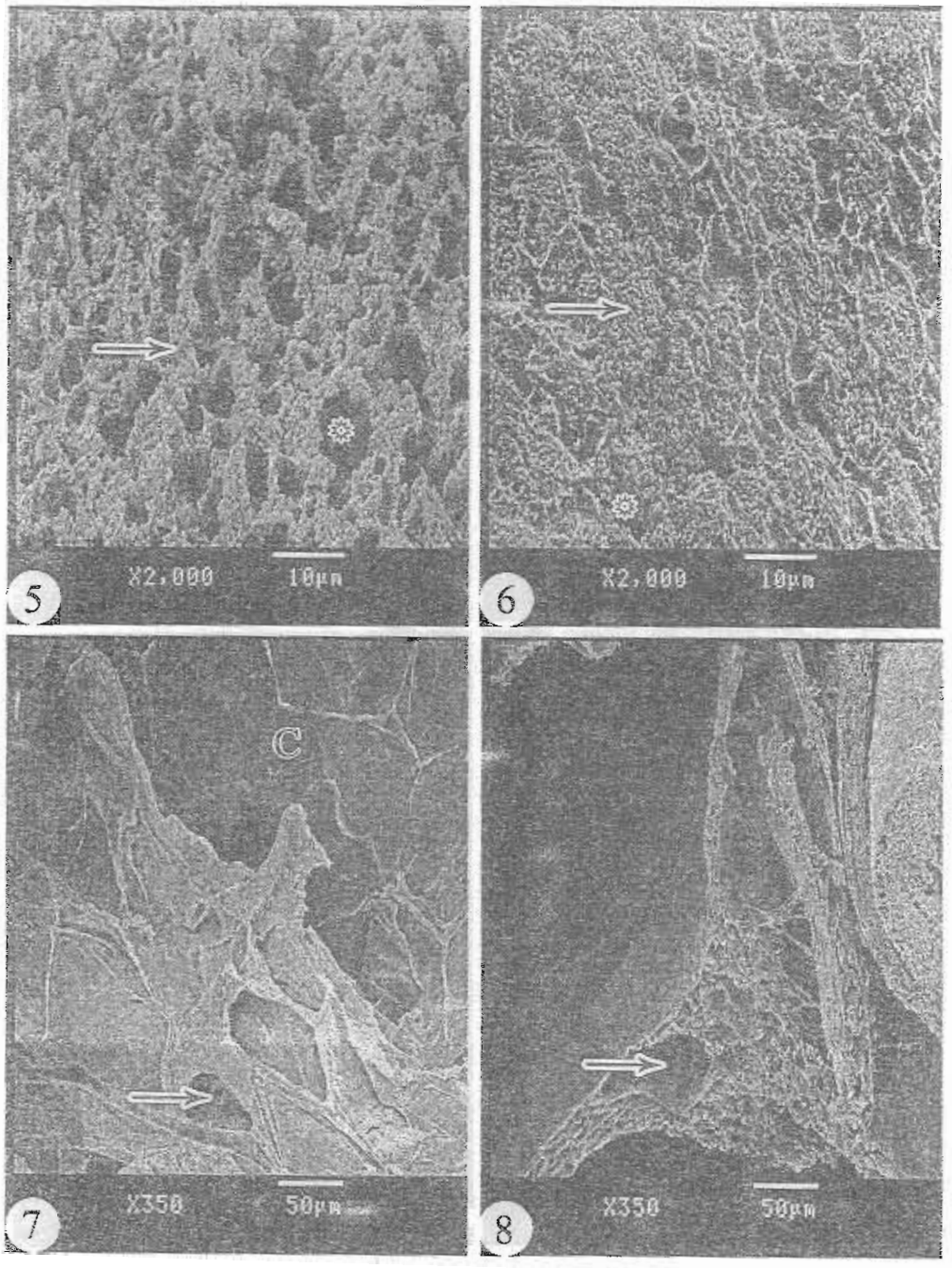
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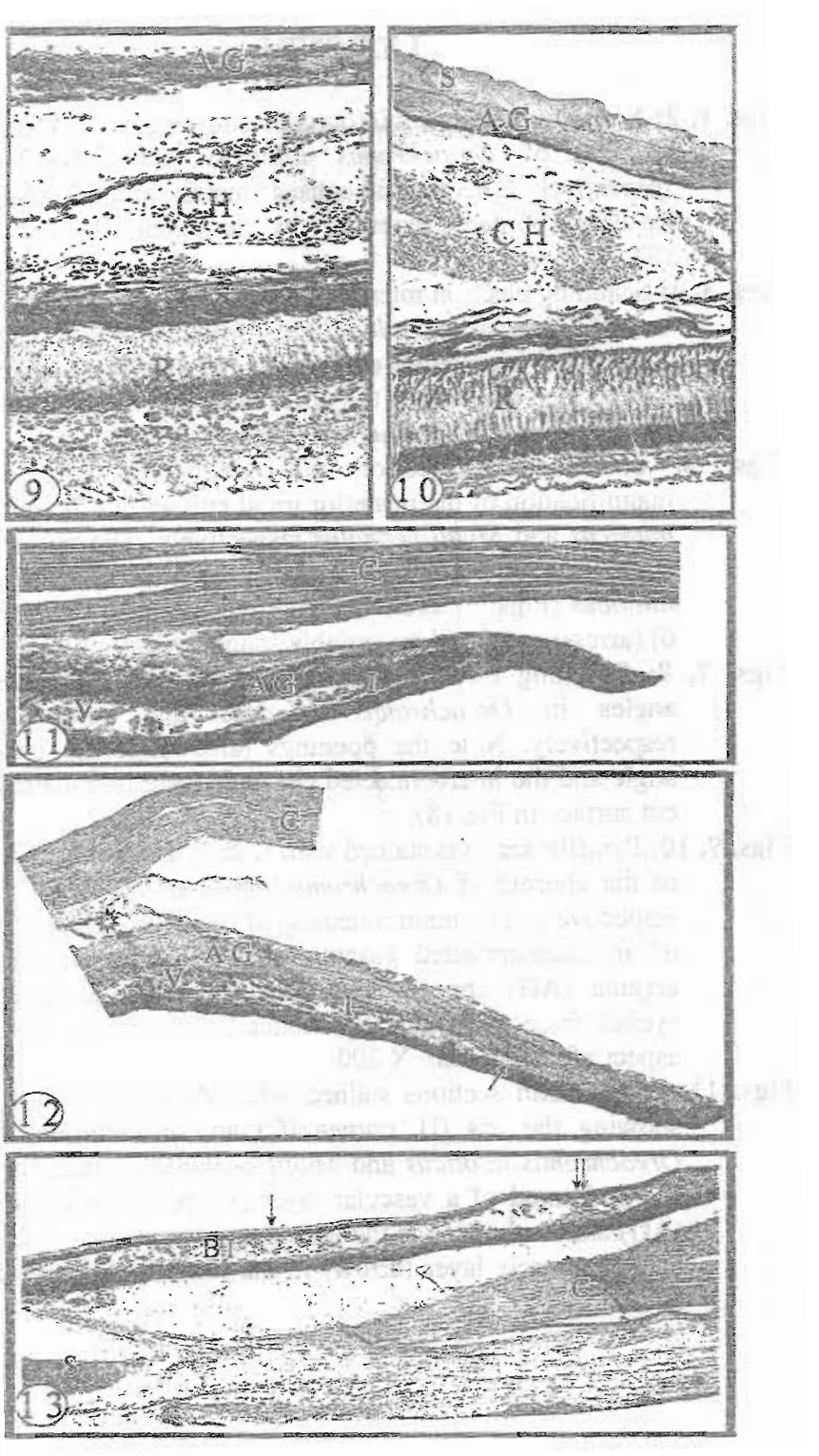
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LEGENDS

Figs. 1, 2: Scanning electron micrographs showing a cut surface of the choroid of *Oreochromis niloticus* and *Mugil cephalus* respectively. The choroid consists mainly of a vascular network (asterisk) bordered externally by the argenta. Notice the sclera (S).

Figs. 3, 4: Scanning electron micrographs showing the *Ora serrata* (OS) in both *Oreochromis niloticus* and *Mugil cephalus* respectively. The retinal pigmented epithelium (RE) shows long processes that shorten abruptly at the ora serrata then slightly elongate on the posterior iridal epithelium (PIE).

Figs. 5, 6: Scanning electron micrographs showing a higher magnification of the posterior iridal epithelium in *Oreochromis niloticus* and *Mugil cephalus* respectively. The posterior iridal epithelium carries relatively long processes in *Oreochromis niloticus* (Figs. 5) and short microvilli in *Mugil cephalus* (Fig. 6) (arrow) separated by variably shaped openings (asterisk).

Figs. 7, 8: Scanning electron micrographs showing the iridocorneal angles in *Oreochromis niloticus* and *Mugil cephalus* respectively. Note the openings (arrow) of the iridocorneal angle and the interconnected channels (asterisk) visualized in a cut surface in Fig. (8).

Figs. 9, 10: Paraffin sections stained with H & E in the posterior portion of the choroid of *Oreochromis niloticus* and *Mugil cephalus* respectively. The main thickness of the choroid (CH) is formed of an interconnected vascular network (*Rete mirabile*). The argenta (AG) appears separating the vascular layer of the eyeball from the sclera (S). Notice the retina (R) on the inner aspect of the choroid. X 200.

Figs. 11, 12: Paraffin sections stained with Masson's trichrome stain showing the iris (I), cornea (C) and iridocorneal angle of *Oreochromis niloticus* and *Mugil cephalus* respectively. The iris is formed of a vascular layer (V) posteriorly and argenta (AG) anteriorly. Notice the iridocorneal angle (asterisk) and the smooth muscle layer (arrow) in the iris of *Mugil cephalus*. X 200.

Fig. 13: Paraffin section stained with H & E showing the base of the iris (BI) in *Mugil cephalus* where it is covered by a bilayered epithelium (arrow) posteriorly, but anteriorly the inner non-pigmented layer fades out and only the pigmented epithelium remains (double arrows). Notice the cornea (C) and the sclera (S). X 200.

DISCUSSION

The two examined fish species inhabits two different watery media with different light intensities (fresh water for *Oreochromis niloticus* and brackish water for *Mugil cephalus*). These different habitats influence the structural criteria of their visual apparatus. Although the vascular coats of the two examined species are similar in general structure, they show some peculiar structural characteristics for each species.

The choroid of both *Oreochromis niloticus* and *Mugil cephalus* consists mainly of a vascular layer bordered externally by a more or less uniform layer of fusiform or rod shaped cells (argenta) that stain light brown in colour. This colouration has been owed to minute crystals in the cells of which it is composed (Parker and Haswell, 1995). The argenta was demonstrated in many fish species where it is generally defined as a reflective layer located in the superficial scleral region of the choroid and not conductive to eye shine because of the presence of melanophores (Nicol, 1980). The above mentioned reflective function may be impaired by the abundant pigmentation demonstrated in the argenta of *Oreochromis niloticus* and may be attributed to the shiny habitat of this fish species. It has been also noted that the fish argenta may also participate in gas regulation of the choroidal region (Lanzing and Wright, 1981).

The vascular layer of the choroid in the examined fish species widens posteriorly forming an interconnected vascular plexus. Grizzle and Rogers (1976) as well as Lanzing and Wright (1981) described this vascular plexus as a thick ring shaped structure and named it the choroid gland. The latter authors added that it is not glandular but is a complex network of blood vessels or Rete mirabile. An intracranial Rete mrabile participating in the vascularization of the brain has been demonstrated in many mammals (Raghavan and Kachroo, 1964 in ox; Ghoshal and Khamas in pig, 1985; Zayed, 1988 in camel). The intracranial Rete mirabile has been suggested to play a haemodynamic role in the

regulation of the cerebral circulation. The pulsation of the retial arteries may assist in returning back of the venous blood (Daniel *et al.*, 1953; Baldwin and Bell, 1963; Kanan, 1970). In our opinion, the presence of such rete in the eye of fishes may serve in two directions. In one direction, it may maintain an adequate supply of blood to the neighbouring retina particularly in the underwater high pressure habitat. In the second direction it may assist in returning venous blood to the general circulation.

The current study agrees with previous statements on other fish species (Duke-Elder, 1958: Prince, 1956; Lanzing and Wright, 1981) that the vascular tunic has no ciliary body. However the vascular tunic of both examined fish species was covered internally, at the root of the iris by bilayered epithelium; an inner non pigmented and an outer pigmented layer. This region has been described by the latter authors as the retinal component of the iris which is covered by ciliary epithelium. It can be suggested that this region may represent the remnant of the ciliary body in fishes.

Our study shows that the iridal argenta of *Oreochromis niloticus* demonstrates melanin pigmentation, a character which is not seen in *Mugil cephalus*. This pigmentation may help in absorption of excessive light rays as an accommodation to the shinny habitat of *Oreochromis niloticus*. The *Mugil cephalus*, however devoid of this pigmentation and this may amplify the reflective power of the argenta in this species which inhabits a brackish water.

The present investigation reveals that the iris of *Mugil cephalus* has a thin layer of smooth muscle fibers near its pupillary margin. This layer is absent in *Oreochromis niloticus*. A previous studies on the iris of Pseudomugil signifer ascertained the absence of smooth muscles (Lanzing and Wright, 1981). However, Romer and Parsons (1978) mentioned that smooth muscles are present in the iris of shark, some teleosts and tetrapods. It is well known that the presence of smooth muscles in the iris enables it to control the amount of light entering the eye through the pupil. *Mugil cephalus* as a brackish water inhabitant may be equipped with these iridal muscles to dilate the pupil in areas with dim light.

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