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Structure and function of the retinal pigment epithelium, photoreceptors and cornea in the eye of *Sardinella aurita* (Clupeidae, Teleostei)

Mostafa Ali Salem

Zoology Department, Faculty of Science, Zagazig University, Egypt

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KEYWORDS

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Rods;
Cones;
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Cornea;
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Abstract The structure of the pigment epithelium, photoreceptors and the cornea in the eye of a teleost, *Sardinella aurita* was examined by light and electron microscopy. The retinal pigment epithelium forms a single layer of cells joined laterally by cell junctions. Centrally in the retina these cells are columnar, while more peripherally they become cuboidal in shape. The basal (scleral) border of the pigment epithelial cells is not infolded but is relatively smooth. Phagosomes containing lysosome-like bodies are also common features of the retinal pigment epithelium. Numerous melanosomes (pigment granules) are abundant throughout the epithelial cells. These melanosomes probably absorb light which has passed through the photoreceptor layer. Four photoreceptor cells were identified; rods, long single cones, short single cones and double cones. The presence of these types suggests a diversity of photoreceptor function. Square mosaic pattern of cones and well-developed choroid gland are also main features of the eye. The inner segment of rods and cones were rich in organelles indicating much synthetic activity. Calycal processes projecting from cone outer segments are also observed. The cornea includes an epithelium with a complex pattern of surface microplicae, a basement membrane, dermal stroma, an iridescent layer, scleral stroma, Descemet's membrane and endothelium. The autochthonous layer which is seen in some teleosts has not been observed in the cornea of this species. These and other observations were discussed in relation to the photic environment and habits of this fish.

Introduction

Fish and other aquatic animals live in a different light environment than terrestrial species. Therefore, fish possess various kinds of sense organs and use them to detect many kinds of information in the ambient environment. Ali and Klyne (1985) classified the sense organs in fishes into three groups; (1) organs of chemical sense which comprise the olfactory organs, taste buds and Jacobson's organ, (2) organs of vision which include the eyes, and (3) organs detecting pressure change and the movement of the medium which comprise the inner ear, lateral line organs and pit organs. The eye of the fish has been chosen as a focus, in an effort to illustrate how aquatic animals can overcome visual barriers.

E-mail address: salemlab3@hotmail.com

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The eyes of vertebrates show many adaptations that can be related to specific visual tasks or the intensity and spectral composition of light in which species are active (Beaudet and Hawryshyn, 1999). The teleost fishes have been extensively studied and, due to the diversity of light environments and habitats in which they are found, have provided numerous examples of visual specialization in optics, retinal structure, and physiology (Collin, 1997). It has been reported that vision in fish depends on: size and position of the eyes, morphology and types of retinal photoreceptors, structure of the pigment epithelium, structure of the cornea and lens, and on the visual pathways and integrating areas in the brain (Zaunreiter et al., 1991; Schmitt and Dowling, 1999). In the present study, the cornea, the retinal pigment epithelium and the photoreceptors were chosen.

In most vertebrates, the cornea plays an important role in providing protection for the inner structures of the eye and acts as a light collector in refracting incident light into the retina. The fish cornea does not have such a large refractive index relative to the surrounding water medium. However, it still constitutes a protective cover for the eye and provides an optically smooth surface and a transparent window. In comparison with the mammalian cornea many specializations have been reported. These include spectacles and corneal filters (Kondrashev et al., 1986), iridescent layers (Lythgoe, 1976; Collin and Collin, 1998), and an autochthonous layer (Jermann and Senn, 1992), all of which are thought to provide some visual advantage to the animal.

Although the retinal structure is basically the same as in vertebrates, the teleostean retina has been, and still is, a focus for the attention of many researchers due to a number of features that characterize it. Of these features are: (1) the presence of retinomotor movements in response to changes in light conditions (Donatti and Fanta, 2007), (2) the presence of large photoreceptor outer segments and prominent ellipsoids to improve absorption of light (Reckel and Melzer, 2003), (3) the existence of double cones which increase the area available for the absorption of light (Shand, 1997), (4) regular cone mosaic pattern (Reckel et al., 2002), (5) the existence of a well developed retinal pigment epithelium (Braekevelt, 1982), (6) presence of foveae or areas with an increase in photoreceptors and other neurons (Wagner, 1990), and (7) a marked synaptic plasticity as is demonstrated by the formation of spinules (Schmitz and Kohler, 1993) during light adaptation and their disappearance during dark-adaptation, and others. In summary, these features demonstrate the importance of vision in the mode of life and survival of the species.

In contrast to many other vertebrates, the teleost retinal structure often varies distinctly between the allied families, sometimes even between genera within a family (Ali and Klyne, 1985). It contains highly specialized types of photoreceptors, i.e., rods, single long and short cones, double cones and triple cones, that are arranged in distinct row, square or hexagonal patterns (Reckel et al., 2002; Darwish et al., 2015). The interspecific variations in retinal structure reflect the feeding habits and photic habitat conditions of the respective species. Rod cells provide high visual sensitivity, being used in low light conditions, while cone cells provide higher spatial and temporal resolution than rods and allow for the possibility of color vision by comparing absorbances across different types of cones which are more sensitive to different wavelengths (Flamarique and Harosi, 2000; Al-Adhami et al., 2010). The ratio of rods to cones depends on the ecology of the fish species concerned, e.g., those mainly active during the day in clear waters will have more cones than those living in low light environments (Wagner, 1990; Collin et al., 1996).

A basic structural plan seems to be common to all vertebrate photoreceptors with the typical photoreceptor consisting of an outer segment (light-capture area) joined to an inner segment (synthetic area that is often further subdivided into compartments) by a non-motile cilium, a nuclear region, and a synaptic end piece (Rodieck, 1973). In addition, in a number of teleosts a well-defined repeating mosaic in the arrangement of the cone photoreceptors has been reported (Garcia and De Juan, 1999; Reckel and Melzer, 2003; Salem, 2004).

The retinal pigment epithelium normally consists of a single layer of cuboidal to low columnar cells forming the outermost (scleral) layer of the neural retina. The retinal pigment epithelium is an essential layer of the vertebrate retina charged with several specialized roles indispensable to the visual process and as such has been described in a variety of teleost species (Es-Sounni and Ali, 1986; Braekevelt et al., 1998; Donatti and Fanta, 2007; Darwish et al., 2015). The pigment epithelium along with the choriocapillaris and Bruch's membrane is intimately involved in several processes vital to the proper functioning of the photoreceptor cells and hence to vision itself. Among the best known functions of the pigment epithelium are: (1) the storage and modification of vitamin A precursors of the visual pigments (Braekevelt et al., 1998); (2) the architectural support and proper orientation of the photoreceptor outer segments during light and dark adaptations (Bernstein, 1961); and (3) the selective transport of materials to and from the photoreceptors (Es-Sounni and Ali, 1986). This layer is normally pigmented to absorb light which has passed through the photoreceptor layer.

The round sardinella, *Sardinella aurita* (Clupeidae, Teleostei) is a mid-sized pelagic fish that represents one of the most important commercial fishery resources in the Egyptian Mediterranean Sea (Madkour, 2011) and one for which no TEM data on the eye are currently available. Therefore, the purpose of this study was to investigate the morphology and fine structure of the retinal pigment epithelium, photoreceptors and cornea in the eye of *S. aurita* to provide more data of the vision of a predatory fish in order to increase our understanding of the morphology and structure of the eye and its relation with the ecology of the species.

Materials and methods

The specimens of the round sardinella, *S. aurita* were obtained from Mediterranean Sea at Port Said coasts. The extraocular muscles were cut and the eyes were excised, and following the removal of the lens and vitreous, small pieces from the cornea and various regions of the retina were cut and fixed in 2% glutaraldehyde in 0.1 M phosphate buffer for 2 h and post-fixed in 2% osmium tetroxide in 0.1 M phosphate buffer for 1 h. Tissues were then dehydrated in ascending ethanol series and embedded in epoxy resin. Some thick sections were cut by the Porter-Blum ultramicrotome using glass knife and stained with toluidine blue. For transmission electron microscopy observations, thin sections were cut and stained with lead citrate and uranyl acetate and examined under a JEOL 100 CX transmission electron microscope at 80 kV.

For light microscopic examinations, other pieces from the cornea and the eye were fixed in Bouin's solution, dehydrated in increasing ethanol series and embedded in paraffin wax. The samples were cut at 2–3 microns and stained with haematoxylin and eosin stain.

Results

Light microscopy observations

S. aurita has spherical eyes locate laterally. The eye contains thick pigment epithelium (Fig. 1), transparent cornea and a well developed choroid gland (Figs. 2 and 3). The pigmented epithelium lies between the choroid layer and the neural retina where the processes of their cells reach the outer segments of the photoreceptor cells (Figs. 1 and 3). It is formed of a single layer of heavy pigmented columnar cells (Figs. 1 and 3). Most of the pigment granules are more concentrated at the central part of the retina and their color is brown due to the presence of melanin (melanosomes).

The retinal layers that include the photoreceptor layer are clearly differentiated and seemed to be thick at the temporal region of the eye (Figs. 2 and 3). A large choroid gland (choroids rete mirabile) lying between the sclera and the pigment epithelium is observed (Fig. 3). The retina of *S. aurita* contains four basic types of photoreceptor cells; long single cones, short single cones; double cones and rods (Fig. 4). The nuclei of cones and rods are concentrated in a distinctive layer called the outer nuclear layer (Fig. 4). In cross section, different cones in the retina are arranged regularly as a mosaic pattern where each pattern of the mosaic consists of four double cones surrounding a single cone (Fig. 5).

The cornea consists of three main layers; the corneal epithelium, dermal stroma and iridescent layer (Fig. 6). The stroma contains several layers of collagenous tissue forming thick lamellae (Fig. 6). The iridescent layer formed of several plates of collagen fibrils (Fig. 6). The stroma separated from the

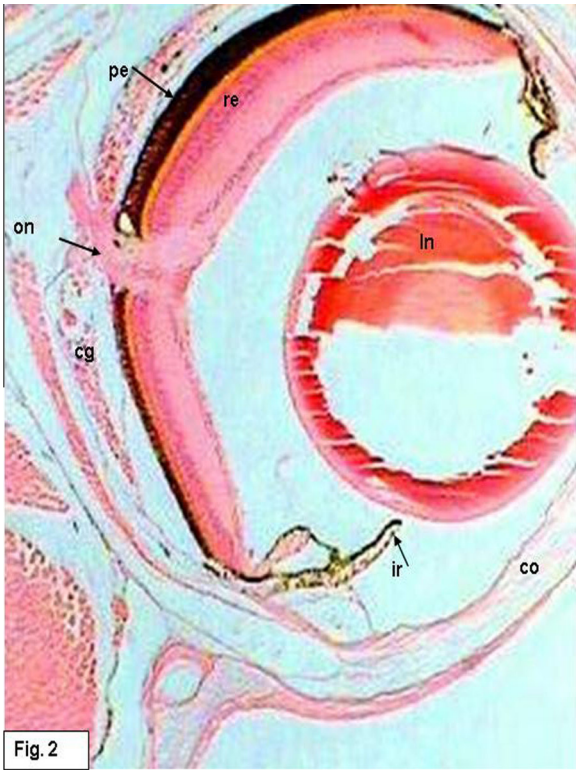

Figure 2 Light micrograph of a transverse section through the eye showing different components of the eye. cg, choroid gland; co, cornea; ir, iris; ln, lens; on, optic nerve; pe, pigment epithelium; re, retina ×225.

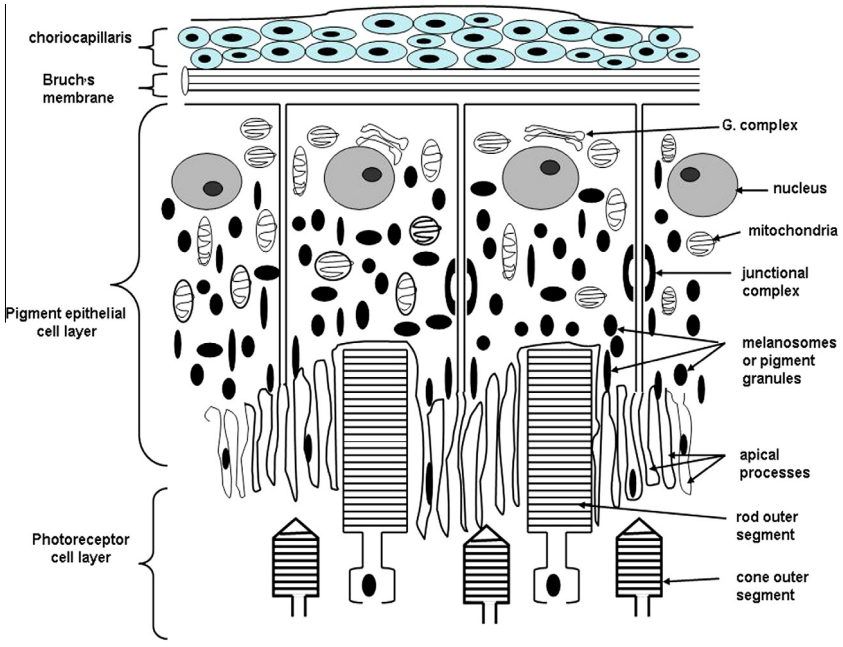

Figure 1 Schematic drawing of the pigment epithelium of *Sardinella aurita* in transverse section showing structure of its cells and the relation with the neighbouring layers.

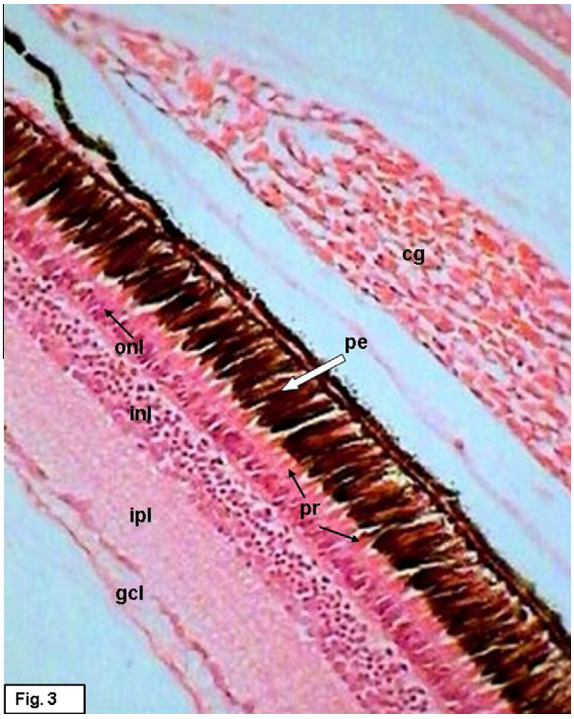

Figure 3 Light micrograph of a transverse section through the eye showing the thick pigment epithelium (pe), a well developed choroid gland (cg) and the photoreceptors (pr) which interspersed between the pigment epithelial cells. Note the other retinal layers: gcl, ganglion cell layer; inl, inner nuclear layer; ipl, inner plexiform layer; onl, outer nuclear layer ×400.

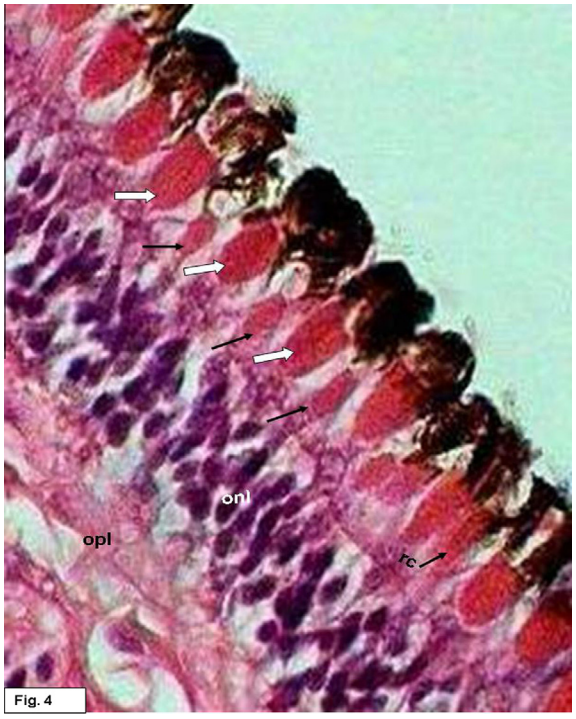

Figure 4 Light micrograph of a transverse section through the retina showing different photoreceptor cell types which include single cones (black arrows), double cones (white arrows) and rod cells (rc). onl, outer nuclear layer (nuclei of the photoreceptor cells); opl, outer plexiform layer ×550.

iridescent layer by a layer of granular material. The stroma and iridescent layer form the main bulk of the cornea. On the posterior surface of the cornea are several layers, the details of which are difficult to distinguish with light microscopy and will describe by electron microscopy.

Electron microscopy observations

The pigment epithelial cells of *S. aurita* are tall columnar cells (Fig. 7). They form a single layer between Bruch's membrane basally (sclerally) and the photoreceptor cells apically (vitreally) and joined laterally near the basal borders by cell junctions (Figs. 1 and 8). The basal border of these cells, which in many species is highly folded, is here seen to be relatively smooth (without foldings) (Fig. 7). Numerous mitochondria with various sizes and shapes are scattered throughout the basal region of the epithelial cells (Figs. 7 and 8). The pigmented epithelial cells display a flattened hexagonal shape in cross-section and are contiguous with 6 other epithelial cells (Fig. 9). The oval to spherical vesicular nucleus is located near the mid-region of the epithelial cells (Fig. 9). Phagosomes containing lysosome-like bodies and lipid droplets are also common features of the retinal pigment epithelium of this species (Fig. 10). Numerous melanosomes (pigment granules) are abundant throughout the pigment epithelial cells (Figs. 1 and 10).

Bruch's membrane, the boundary between the retina and choroid, may appear as a relatively simple layer of homoge-

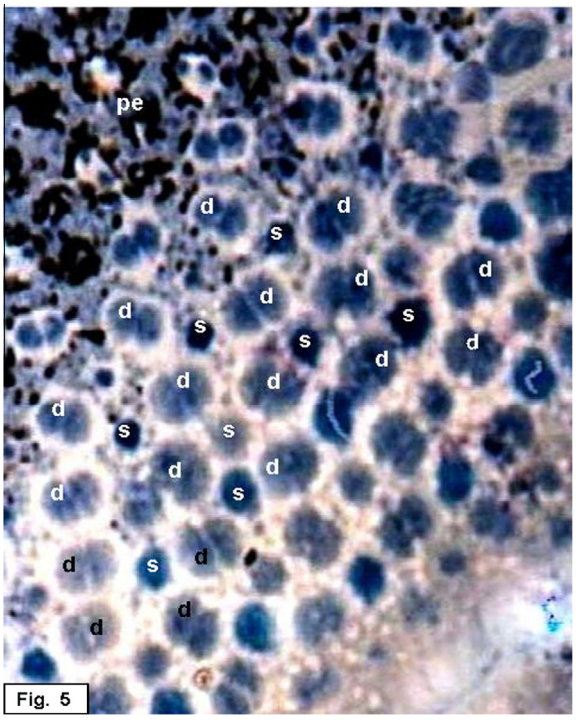

Figure 5 Light micrograph of a cross section through the retina showing the square mosaic patterns, each consists of four double cones (d) surrounding a single cone (s) ×600.

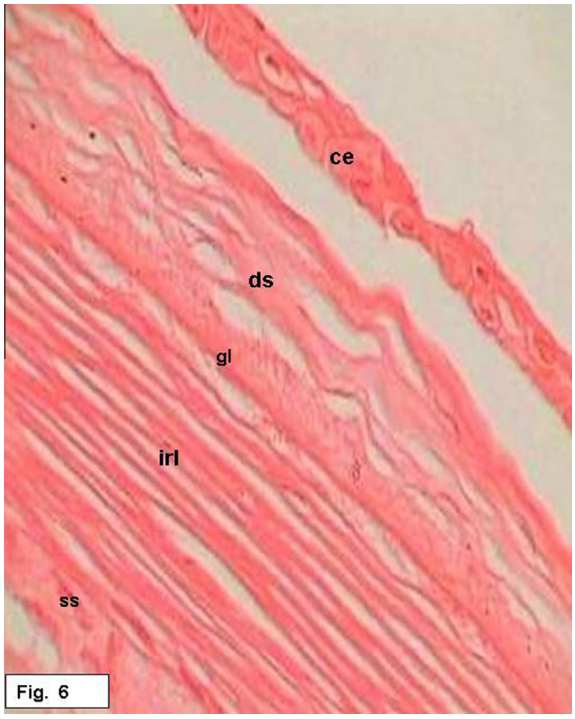

Figure 6 Light micrograph of a transverse section through the cornea showing arrangement of the different layers in the cornea. ce, corneal epithelium; ds, dermal stroma; gr, granular layer; irl, iridescent layer; ss, scleral stroma ×740.

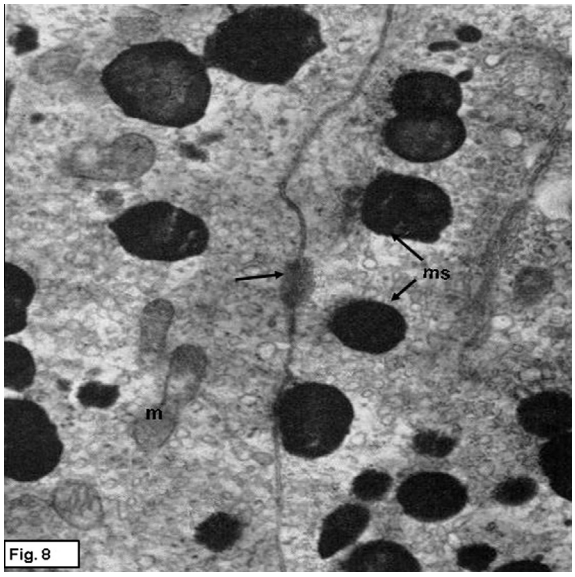

Figure 8 Electron micrograph of the mid region of the pigment epithelium in the central retina. A cell junction (arrow), melanosomes (ms) or pigment granules and mitochondria (m) are indicated ×13,000.

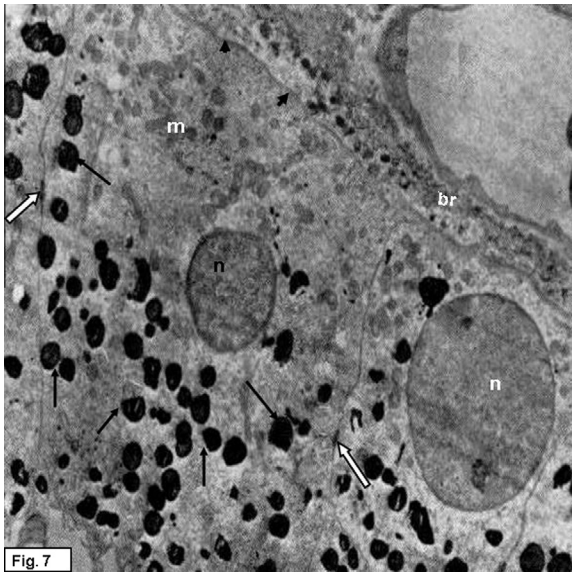

Figure 7 Electron micrograph of the basal (scleral) and mid region of the pigment epithelium in the central retina. The basal region of the epithelial cells (arrowheads) is relatively smooth. Cell junctions (open arrows) and two epithelial nuclei (n) are indicated. Mitochondria (m) are abundant in the basal cell region. Melanosomes or pigment granules (arrows) are rich in the mid and apical region of the epithelial cells. br, Bruch's membrane ×11,500.

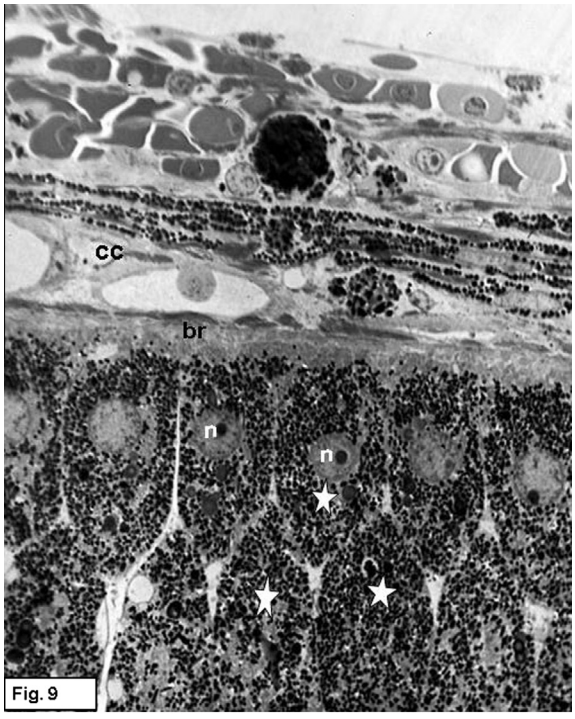

Figure 9 Electron micrograph of cross-section of pigment epithelial cells in the basal (scleral) region. Note hexagonal shape of pigment cells (asterisk). Choriocapillaris (cc) overlying the Bruch's membrane (br) is indicated. n, nucleus of pigment epithelial cell ×4600.

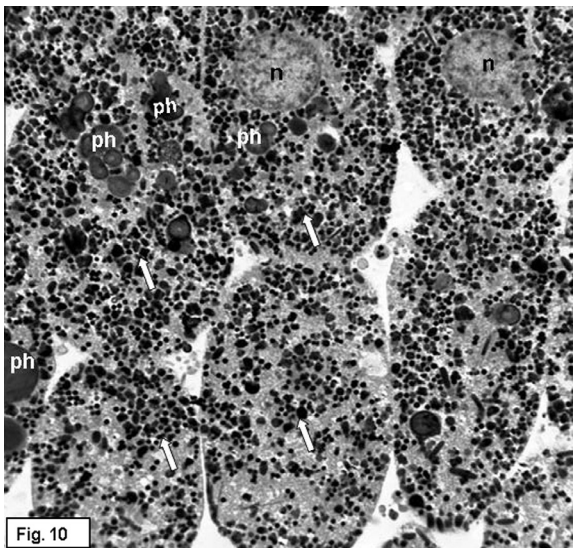

Figure 10 Electron micrograph of cross-section of pigment epithelial cells showing phagosomes (ph) and melanosomes (open arrows). n, nuclei of pigment epithelial cells ×8400.

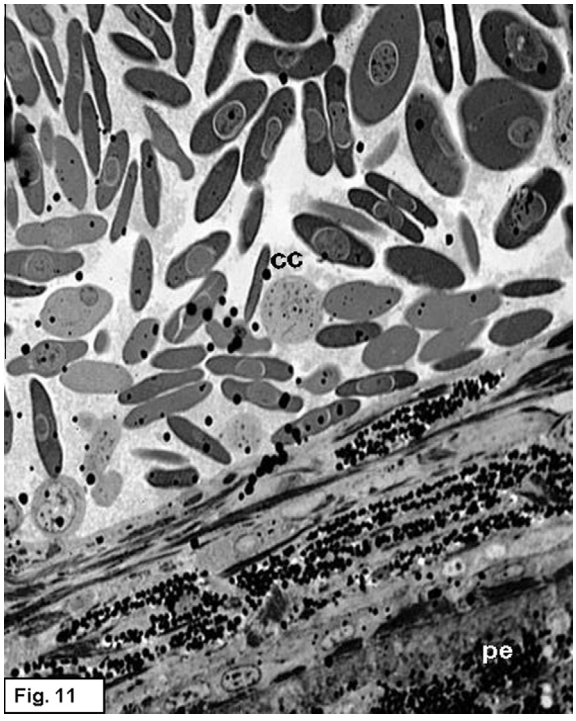

Figure 11 Electron micrograph of the choroidal region showing the choriocapillaris (cc) overlying the pigment epithelium (pe) ×5400.

neous material (Figs. 7 and 9). The choroid body, a well-vascularized region located roughly around the point of entry of the optic nerve, is composed mainly of a dense concentration of capillaries (Fig. 11). The afferent and efferent capillaries are aligned parallel to one another and form a rete mirabile (Copeland, 1971; Copeland and Brown, 1977) that is connected with the layer of blood vessels constituting the choriocapillaris.

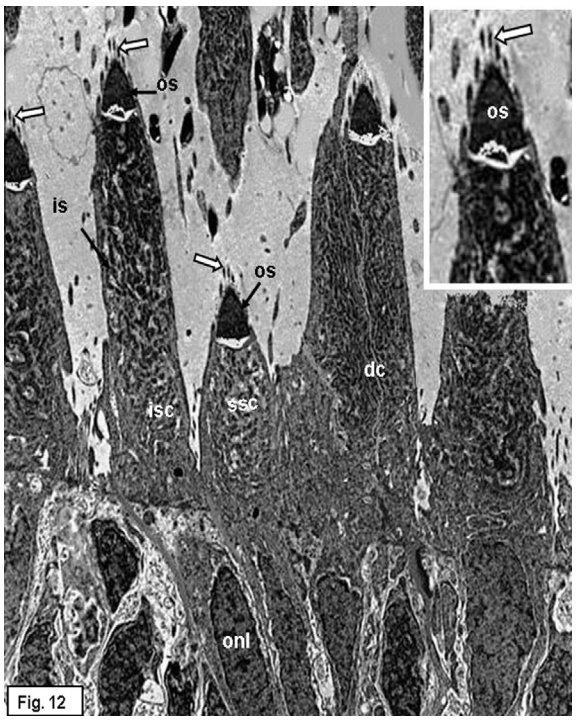

Figure 12 Electron micrograph of cone photoreceptor types to illustrate the long single cone (lsc), short single cone (ssc) and double cone (dc). The outer (os) and the inner (is) segments of cones, calycal processes (open arrows) and outer nuclear layer (onl) containing photoreceptor nuclei are indicated (×10,000). Inserted figure is a magnified part showing calycal processes (open arrows) projecting from cone outer segment (os) (×11,200).

Altogether, four morphologically different photoreceptor cells occur in the retina of *S. aurita*: long single cones, short single cones, double cones and rods (Fig. 12). Double cones are symmetrical, with two halves of the inner segment partly fused (Fig. 12). Their nuclei are elongated and lie immediately under the outer limiting membrane. The long single cone has a large inner segment, while the inner segment of the short single cones is half the size of the inner segment of the long single ones (Fig. 12). The outer segments of the double and single cones are short structures extending from the outer end of the internal segment of each cone towards the pigmented epithelium. Cone inner segments (ellipsoids) are filled with numerous mitochondria (Fig. 13). Approximately 8–10 calycal processes, projecting from the cone outer segments, are observed (Fig. 12). Cross sections in the region of the photoreceptor inner segments showed a mosaic pattern. Each pattern of the mosaic consists of four double cones surrounding a single cone (Fig. 13).

The rods of *S. aurita* are thinner and longer cells than the cones and show uniform shape and size of inner and outer segments (Fig. 14). The outer segments are slender structures that reach the pigment epithelial cells. They are rare in number and appeared to be occurred in groups (Fig. 14). The rod inner segment displayed a small group of mitochondria (the ellipsoid). No regular rod mosaic was found within the photoreceptor layer.

The cornea of *S. aurita* is considerably thicker in the center than in the periphery. There is a corneal epithelium consisting

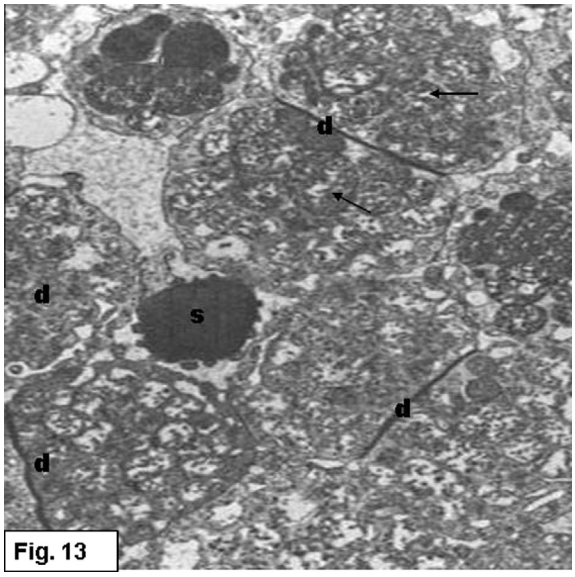

Figure 13 Electron micrograph of a cross-section through the photoreceptor inner segments showing a square mosaic pattern. Note each single cone (s) surrounded by four double cones (d). Mitochondria (arrows) in the cone inner segments are indicated ×11,300.

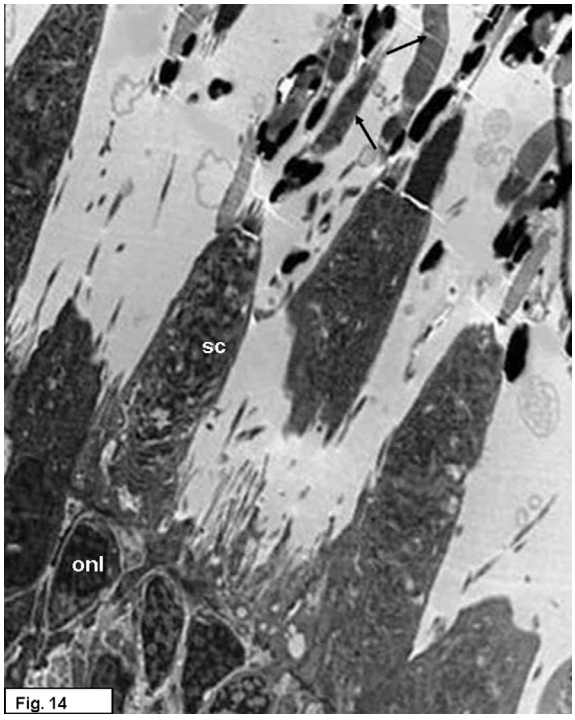

Figure 14 Electron micrograph of photoreceptor region showing rod cells (arrows) and single cones (sc). onl, outer nuclear layer ×8800.

of three layers of cells (Fig. 15). The surface of the epithelium is characterized by the presence of microplicae or microridges (Fig. 15). The large basal cells and the flatter superficial epithelial cells of the cornea are joined with numerous attachment devices including desmosomes (Fig. 16).

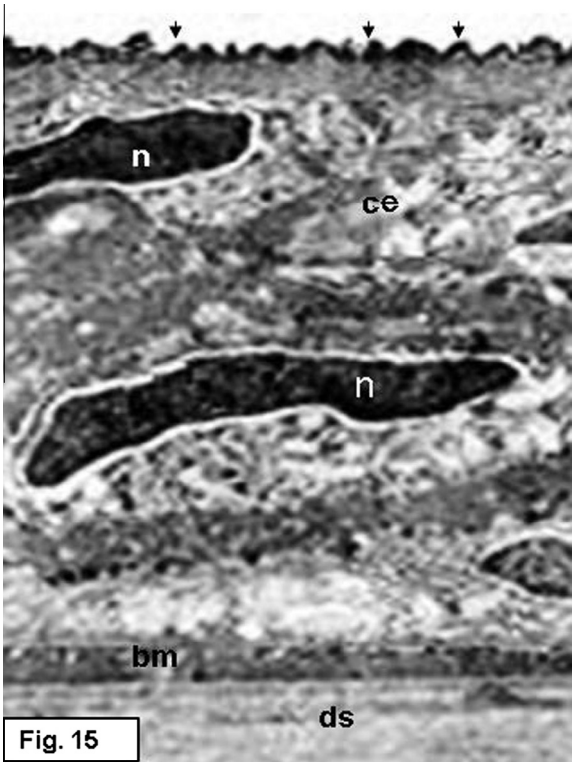

Figure 15 Electron micrograph of the cornea showing microplicae or microridges (arrows) over the surface of the corneal epithelial (ce). The epithelium overlies a granular basement membrane (bm) and a multi-layered dermal stroma (ds). n, nuclei of the corneal epithelial cells ×10,300.

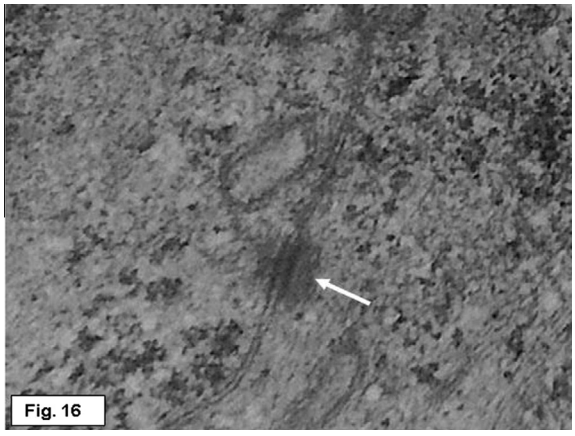

Figure 16 Electron micrograph of the corneal epithelium showing junctional complexes (arrows) between the epithelial cells ×11,500.

The dermal stroma consists of numerous lamellae of collagen fibrils, with flattened cells (keratocytes) occurring between the lamellae (Fig. 17). The anterior lamellae of this layer are thin, while the central and posterior lamellae are thicker. The Bowman's layer is not observed.

Posterior to the dermal stroma is an iridescent layer which is composed primarily of numerous bundles of long, thin, membrane-bound cell processes (Collin and Collin, 1993)

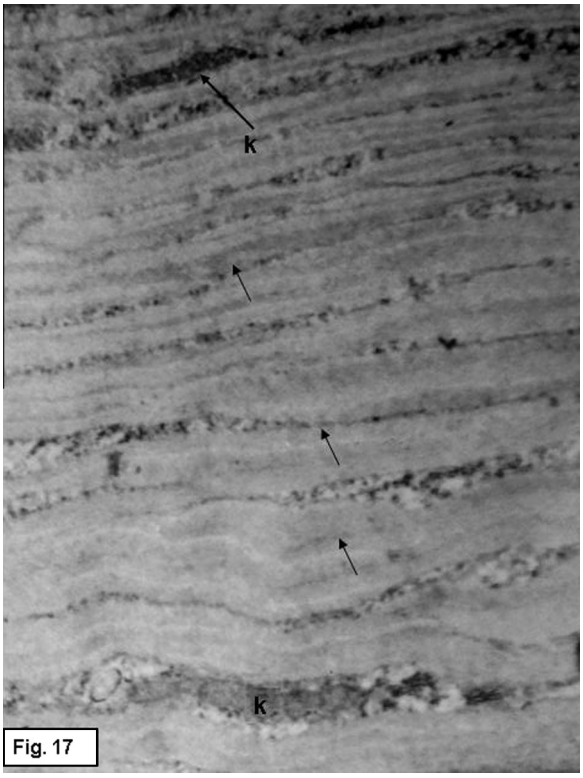

Figure 17 Electron micrograph of the cornea showing the dermal stromal layer. Thin anterior and thick posterior lamellae (arrows) are indicated. Note keratocytes (k) occurring between the lamellae ×10,600.

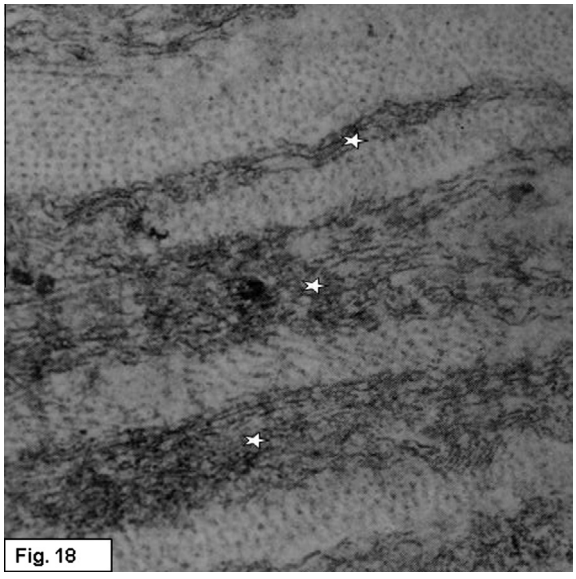

Figure 18 Electron micrograph of the cornea showing the iridescent layer that comprising bundles (asterisks) of membrane-bound cell processes interspersed with collagen lamellae ×13,000.

containing few scattered organelles (Fig. 18). The cell processes are parallel with the corneal surface and have a few thin lamellae of collagen fibrils scattered irregularly between the

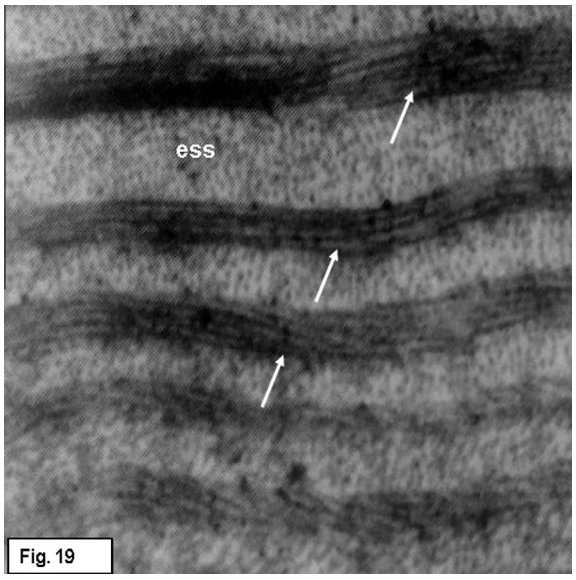

Figure 19 Electron micrograph of the cornea showing arrangement of collagen fibrils (arrows) in the external part of scleral stroma (ess). Note the absence of keratocytes ×13,400.

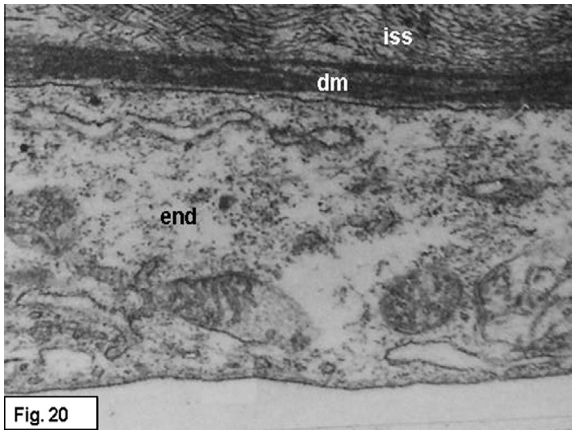

Figure 20 Electron micrograph of the cornea showing the internal part of scleral stroma (iss) and the endothelium (end) overlying by the Descemet's membrane (dm) ×13,400.

bundles. Behind the iridescent layer is the scleral stroma which consists of two parts; an external scleral stroma and internal scleral one. The external scleral stroma contains numerous lamellae of parallel collagen fibrils (Fig. 19), while the internal one contains randomly collagen fibrils overlying the Descemet's membrane (Fig. 20).

There is a prominent Descemet's membrane extending to the periphery of the cornea (Fig. 20). The corneal endothelium which consists of a single layer of cells is present behind Descemet's membrane (Fig. 20). There is no autochthonous layer in this species.

Discussion

S. aurita is highly active fish that range over wide areas in tropical and sub-tropical regions and swim for considerable

distances (Abdel Aziz and Gharib, 2007). Therefore, they continuously face a high variable biotic and abiotic environment. They possess a complex behaviour that requires a complex visual system to face all situations. They have relatively big eyes that show mobility, increasing their visual field what is useful for swimming and searching for food.

S. aurita has a conspicuous retinal pigment epithelium that occupies approximately one third of the visual retina. This is useful for the protection of the outer segments of the photoreceptor cells and avoiding the bleaching of the photopigment present in this region.

Although certain specific differences and specialization are noted, the morphology of the retinal pigment epithelial layer of the *S. aurita* is quite typical of that described for most other vertebrates (Rodieck, 1973; Nilsson, 1978). The basal (scleral) border of the retinal epithelial cells is highly infolded in some fishes and this is believed to indicate an active role in fluid transport by these cells (Dowling and Gibbons, 1962). In *S. aurita*, however, this basal region is relatively smooth. Such observation has previously been reported in other fish retinas (Okuda, 1962; Braekevelt, 1974) and perhaps indicates a lowered rate or volume of fluid transport by this layer in the retina.

The apical processes of the pigment epithelial cells which intimately enclose photoreceptor outer segments are also a common feature in most vertebrates described (Braekevelt, 1982; El Bakary, 2014). It has been suggested that these apical processes are active in phagocytosis (Young, 1978) as well as imparting architectural stability to the photoreceptor outer segments (Bernstein, 1961). Within the pigment epithelial cells are melanosomes (pigment granules) which are more numerous in the peripheral region of the retina. As in other species these melanosomes would absorb light which has passed through the photoreceptor layer (Moyer, 1969). The movement of melanosomes within the retinal epithelial cells in response to environmental illumination is a common feature in teleosts, amphibians and some birds (Rodieck, 1973; Braekevelt, 1982). Pigment migration is usually coupled with photoreceptor lengthening or shortening and is referred to as photomechanical movements or retinomotor responses (Ali, 1975). It is generally believed that retinomotor responses occur to mask or unmask photoreceptor outer segments so as to adapt the eye for day or night vision.

Phagosomes have been reported in some fish species (Rodieck, 1973; Braekevelt, 1977) and are known to be the phagocytosed portions of photoreceptor cell outer segments which are periodically shed (Young, 1978).

The three types of cones which are observed in the retina of *S. aurita*; double cones, long single cones, and short single cones, have been found in other teleosts (Flamarique and Harosi, 2000; Reckel et al., 2001; Shand et al., 2002; Al-Adhami et al., 2010; Fishelson et al., 2012) where their shape, size, and density differ among different species, being the single cones the basic type of photopic visual cell for all vertebrates. The relation between the presence of certain photoreceptor and the characteristics of the environment is not yet clear. Interspecific comparisons showed that some species that live in shallow waters, like *S. aurita*, have a mosaic pattern of double and single cones. On the other hand, some species that live in deep waters show only single cones, or pure rod retinas (Munk, 1981).

Considering the ecology and the behavioural patterns of *S. aurita*, one can suggest that most certainly the presence of three different types of cones as well as of rods is related to a wide range of perceptions not only of different wave lengths and luminosity but also of shapes, colors, and movements. It has been reported that cone photoreceptors are responsible for photopic vision and provide a higher spatial resolution than rods which are usually responsible for scotopic vision at lower light intensities (Ali and Klyne, 1985; Paulus et al., 1986). As longer cones are considered to respond to longer wave lengths, and shorter cones respond to shorter wave lengths (Munk, 1981), the retina of *S. aurita* will be able to respond to more or less the entire spectrum of wave lengths. From the evolutionary point of view this is a very positive characteristic as it allows high adaptability to unexpected situations, increasing the chances of survival.

In many teleost groups, the different cone morphologies reflect different receptor-specific wavelength sensitivities (Robinson et al., 1993). In some species the dominating elements of the visual cell layer are the double cones (Munk, 1981; Al-Adhami et al., 2003). This is not the case for *S. aurita* where an equal number of double and long single cones are arranged as a rectangle mosaic. It is considered that the density and presence of different types of cones can indicate the level of perception of movements in all directions and it is associated with high acuity vision (Reckel et al., 2002; Darwish et al., 2015). It can be suggested that the density of three different types of cones and the presence of a moderate number of rods contribute effectively to the quick visual reactions to the presence of food. It may also allows migration and swimming for long distances.

The cone photoreceptor mosaic has been reported in a variety of species but appears to be best developed in the shallow water or epipelagic teleost retina (Collin et al., 1996; El Bakary, 2014; Darwish et al., 2015). In the retina of *S. aurita*, the cones are arranged in a regular square mosaic pattern where each single cone is surrounded by four double ones. A regular square mosaic pattern has also been found in *Lepomis cyanellus* (Cameron and Easter, 1995), *Zosterisessor ophiocephalus* (Ota et al., 1994), *Anguilla japonica* (Omura et al., 1997), *Micropterus salmoides* (Garcia and De Juan, 1999), *Coilia nasus* (Haacke et al., 2001) and in mud skipper fish *Periophthalmus barbarus* (Salem, 2004). The rods did not confirm to the regular pattern but they filled the spaces between the cones. A mosaic pattern of cone arrangement is felt to facilitate the retina's ability to gather moving visual stimuli, since species comparisons indicate that active predatory species living in shallow water have well-developed mosaics while deeper dwelling species have less well developed mosaics (Firnald, 1982). Another closely allied potential function for a regular mosaic involving double cones particularly is in the detection of polarized light which would be important in navigating as well as the detection of prey in turbid waters (Cameron and Pugh, 1991). On the other hand, it has been suggested that the square mosaics appear to be common in predatory fishes (Wagner, 1990). In conclusion, the presence of square mosaic pattern including different kinds of cones may indicate a well developed color vision, good movement detection and good visual acuity.

The photoreceptor cells are extremely metabolically active cells (Collin, 1997) and it is well documented that both rods and cones constantly renew their outer segments by the addition of new discs basally and the shedding of older discs apically (Young, 1978). In the case of *S. aurita*, the cone outer

segments are short and the distal part of each cone inner segment includes an ellipsoid which contains a huge number of elongate mitochondria. The portion of the inner segment between the ellipsoid and the external limiting membrane is referred to as the myoid region, for this is the region which elongates or shortens in response to environmental lighting in such lower vertebrates as teleosts and amphibians (Rodieck, 1973) and this phenomenon is called retinomotor response (Ali, 1975; Burnside and Laties, 1979). As mentioned above, retinomotor responses are believed to adapt the eye for day or night vision by shielding or exposing photoreceptor outer segments to incident light. Rods would thus elongate and be shielded by the melanosomes of the pigment epithelium which move apically in the pigment epithelial cells in light adaptation, while cones would shorten and better expose their outer segments under the same stimulus. The reverse would occur in dark-adaptation and the melanosomes of the pigment epithelium would also move basally away from the photoreceptor outer segments. This phenomenon of retinomotor responses probably occurs in the *S. aurita* of the present study.

In the present investigation, approximately 8–10 calycal processes projecting from cone outer segments are observed. Morris and Shorev (1967) suggested that the calycal processes may assist in the proper orientation of the outer and inner segments of the photoreceptors, which is thought to be crucial for accurate visual function. They have also been suggested to prevent the photoreceptor outer segments from rotating about the eccentrically situated connecting cilium. Rodieck (1973) proposed that the calycal processes may represent a channel for the uptake of nutrient transfer to the inner segments.

A large choroid gland (choroids rete mirabile) lying between the sclera and the pigment epithelium is observed in *S. aurita*. A similar result has been also found in many teleost fishes, e.g. *Gambusia schoelleri* (Khalil, 1989), *Epinephelus fuscoguttatus* (Salem and Al-Jahdali, 2006) and recently by Foroogh et al. (2014) in the rabbit fish eye (*Siganus javus*). However, the choroid gland is absent in the eye of *Anguilla anguilla* (Wittenberg and Haedrich, 1974). The function of the choroid gland is thought to maintain a large pressure of oxygen at the retina and hence make transport from the choriocapillaris to the retinal pigment epithelium (Wittenberg and Haedrich, 1974). It can also act as a cushion against compression of the eye ball.

The cornea of *S. aurita*, as examined by light and electron microscopes, seemed to compose of six main layers: the corneal epithelium, dermal stroma, iridescent layer, scleral stroma, Descemet's membrane and the endothelium. This result has been also recorded in a moderate number of fishes, e.g. *Coryphoblennius galerita* (Jermann and Senn, 1992); *P. barbarus* (Salem, 2004). The corneal epithelial cells are interconnected by numerous desmosomes, which probably provide mechanical integrity by anchoring intermediate filaments to the sites of adhesion at the cell membrane, and thus play a crucial role in the maintenance of tissue architecture.

The outer surface of the corneal epithelium in *S. aurita* appeared to be covered with microplicae or microridges. Similar observations have previously been found in other fishes (Pcheliakov, 1979; Collin and Collin, 2000; El Bakary, 2014). The presence of microplicae may be species specific and they may perform different functions. They probably increase the surface area of the epithelium aiding diffusion and active transport of salts and other solutes. In elasmobranches (Harding et al., 1974), amphibia (Kaltenbach et al., 1980), and mammals (Pfister, 1973; Doughty, 1994), there are no microplicae present on the corneal surface but a dense mat of finger-like protrusions or microvilli occurred. In these groups the microvilli are thought to play a major role in stabilizing the corneal tear film which is essential for clear vision.

The basement membrane of the corneal epithelium in *S. aurita* seemed to be thick, and may help to provide a more effective barrier to the movement of substances in and out of the cornea (Collin and Collin, 1993). The Bowman's membrane with a random arrangement of collagen fibres underlying the basement membrane of the epithelium has been recognized in most elasmobranchs (Keller and Pouliquen, 1988), and in some species of teleosts (Shand, 1988; Zhao et al., 2006) but not all (Collin and Collin, 1988). The Bowman's membrane was not observed in the cornea of the present species. This random arrangement of collagen fibres may be an adaptation to an aquatic environment where, in combination with the corneal epithelium it may act as a corneal barrier to sodium and water movement (Edelhauser and Siegesmund, 1968). The ontogeny and phylogeny of Bowman's layer is not well understood and further researches are necessary to explain its seemingly random development throughout the vertebrate animals.

In *S. aurita*, the cornea is characterized by the presence of dermal and scleral stroma. The latter consisted of an external and an internal stroma. The dermal stroma was thick compared with that found in other teleosts such as *Gambusia affinis* (Lantzing and Wright, 1982) and *P. barbarus* (Salem, 2004), where the stroma was the thinner layer in the cornea. The increased corneal thickness produced by the intercalation of various layers may incur some advantages including providing support and suitable intervention patterns. Collagen fibril plates or lamellae forming a complex layer, originally described by Lythgoe (1971) as an iridescent layer, were observed in the cornea of *S. aurita*. An iridescent layer has also been seen in other teleost fishes, e.g. *Pomatoschistus minutus* (Lythgoe, 1976), *Platichthys flesus* (Pcheliakov, 1979), *Lepidoglaxias salamandroides* (Collin and Collin, 1996). The function of the iridescent layer has been postulated to reduce intraocular flare thereby increasing visual range underwater without sacrificing sensitivity (Lythgoe, 1976). A theory supposed that the function of the iridescent layer with its different orientations may be in the reduction of intraocular glare caused by bright down-welling light or to produce interference by filtering unwanted wavelengths of light from entering the eye at specific angles (Lythgoe, 1976). Other putative functions of the iridescent layer include birefringence, a colored filter, a polarizing filter, camouflage or display and the enhancement or suppression of reflection (Shand, 1988).

The collagen fibrils of the iridescent layer of the shallow-water *S. aurita* and of other fishes as *Nemanthias carberryi* (Locket, 1972) are oriented parallel to the collagen lamellae of the dermal stroma which are oriented in oblique position and thereby is not perpendicular to the bright downwelling light as found in some deep-sea fishes like *Microgadus proximus* (Collin and Collin, 1998). Therefore, the orientation of the iridescent fibrils forms an infinite number of different angles with the dermal stroma and with the surface of the cornea causing interference often producing a colored reflection (Locket, 1972). It is possible that the iridescent layer may not constitute a reflecting surface to reduce intraocular flare

along the visual axis but acts as an anti-reflection device whereby light entering at normal incidence may show destructive interference, assuming the refractive indices of stroma and plates of the iridescent layer are sufficiently different (Lythgoe, 1975).

The Descemet's membrane and the endothelial layer are common structures in the corneas of most vertebrate classes (Collin and Collin, 1998). They were found in the cornea of *S. aurita*, and have been also observed in most fishes, e.g. *Lepisosteus platyrhincus* (Collin and Collin, 1993), *Torquigener pleurogramma* (Collin and Collin, 2000). The Descemet's membrane, however, was absent in the red gurnard *Triglia cuculus* (Lythgoe, 1976) and in the clearnose skate *Raja eglanteria* (Conrad et al., 1994). The thickness of Descemet's membrane and the endothelium is believed to represent the primitive situation in the process of corneal evolution (Margaritis et al., 1976). Moreover, Collin and Collin (1998) revealed that the corneal endothelium in the vertebrates is essential for the maintenance of corneal transparency in a variety of environments, including aerial, terrestrial and aquatic.

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