

# A Comparative Histological Study of the Retina in the Two Types of Iraqi Vertebrates

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**Abstract:** The study has aimed to recognize the histological structure of retina in the eye of two Iraqi vertebrates, the difference of the class, the environment and the nature of nutrition; Iraqi spiny-tailed lizard *Saalaroricata* and white-breasted kingfisher *Halcyon smyrnensis*. The results show that the retina is a vascular in both species, that consists of two main layers: pigmented epithelium and neural layer, which compose of nine layers are: visual cell layer, outer limiting membrane, external nuclear layer, external plexiform layer, internal nuclear layer, internal plexiform layer, ganglion cell layer, optic nerve fiber layer and inner limiting membrane. The pigmented epithelium consists of cuboidal epithelial cells, extending cytoplasmic projection toward visual cells. The visual cell layer in Iraqi spiny-tailed lizard was composed of cones only, while in white-breasted kingfisher was composed of rods and cones. The external plexiform layer is less thick than the internal plexiform layer in both types. The numbers of rows external nuclear layer are less than the number of rows the internal nuclear layer in the studied species. It can be concluded that the differences in the density of visual cells in the species studied are due to increase visual acuity and light level sensitivity.

**Keywords:** retina, histology, Iraqi spiny-tailed lizard, white-breasted kingfisher

## 1. Introduction

The eye of vertebrate can focus light on the cells responsible for the light absorption to form an image of the environment [1]. The vertebrate eye in general with synthetic is similar, to those found in orbits; there are eyeball and parts that help eyelids and several types of glands and muscles [2]. The eyeball has three layers: fibrous layer that contains the sclera and cornea, uvea layer includes the choroid, ciliary body and iris and retina layer [3].

The retina consists of two layers: nine neural layers include photoreceptor (rods and cones) and various neurons and pigmented layer composed of a single row of cuboidal epithelium [4].

Studies have been conducted on reptiles and Iraqi birds [5, 6, 7, 8], but still many aspects that have not been studied, although Iraq's possession of a wide environmental diversity is reflected on biodiversity and to cover some of the scientific aspects of two types of Iraqi vertebrates, these two species were selected on the basis of differences in class, the environment and the nature of feeding them:

Type I: Iraqi spiny-tailed lizard *Saalaroricata* (Blanford, 1874), belongs to Class: Reptilia, order: Squamata, characteristic that head short and broad, body depressed, with a fold on each side of the back are brown, feeding on the scarce vegetation in their desert environment, that predominantly herbivorous [9].

Type II: white-breasted kingfisher *Halcyon smyrnensis* (Linnaeus, 1758), belongs to Class: Aves, Order: Coraciiformes, it is characteristic that back, wings and tail are bright blue. There is a large white spot in throat and breast, beak and foot are red, it is not piscivorous and feeds on insects and reptiles for quality in areas rich in jungles [10].

## 2. Materials and Methods

We collected Iraqi spiny-tailed lizard *Saalaroricata* (Blanford, 1874) and white-breasted kingfisher *Halcyon smyrnensis* (Linnaeus, 1758) from market in the city of Baghdad. Three animals from each species were used in this study.

Anaesthetized animal by chloroform and then dissected of the head, removed the skin, disconnect lower jaw, broken bones of the skull, removed the eyes from orbit and cut muscles their associated. Either the white-breasted kingfisher has adopted the same as in the previous steps as well as the removal of feathers.

The eyeball was fixed in Bouin's solution for 4 hours, cut in cross-sectional acute scalpel to 1/3 and 2/3, then let go in Bouin's solution for 10 hours at room temperature and followed by washing 24 hours with 70% ethyl alcohol. The dehydrated in ascending grades of ethanol alcohol was (80%, 90%, 95% and 100%) about 45 minutes for each concentration, cleared in xylene for 45 minutes and embedded in liquid paraffin wax at 58°C. After obtaining the paraffin blocks and sectioned at (5 µm). The sections are stained for general histological purposes with Harris's Haematoxylin and Alcoholic Eosin (H&E) [Modified from 11]. The selected sections were tested with light microscope and photographed with digital camera (Canon).

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference -LSD test was used to significant compare between means in this study [12].

## 3. Result and Discussion

The results have showed that the retina in the eye of the animals is a vascular, therefore, they provide oxygen and nutrition through blood vessels in the conical papilla in reptiles [2], but birds supply oxygen and nutrition through a

highly vascular, located above the optic nerve and extending to the vitreous humor known as Pectin oculi [13].

The retina consists of two main layers: the external is the pigmented epithelium and the interior is the neural layer divided into nine layers: the visual cell layer consisting of rods and cones, outer limiting membrane, the external nuclear layer, the external plexiform layer, the internal nuclear layer, the internal plexiform layer, the ganglion cell layer, the optic nerve fiber layer and the inner limiting membrane (fig. – 1a, 1b), this is characterized by retina in vertebrates [7, 14, 15]

The pigmented epithelium consists of a single row of cuboidal epithelial cells, with an oval nucleus, based on a basement membrane extending from its surface several cytoplasmic elongations extend between visual cells and characterized by their containment of melanin granules (fig. – 2a, 2b), this layer supports the visual cells and absorbs scattered light that passes through these cells [16].

The neural retina consists of nine layers: visual cells layer consisting of rods and cones, each of these cells consists of an outer segment that interferes with the extend pigment epithelial cells and inner segment. The visual cell layer in Iraqi spiny-tailed lizard is composed of cones only. The outer segment of the cones is conical and wide (fig. – 3a). The visual cell layer in white-breasted kingfisher is composed of cones and rods. The outer segment of the cones is conical and wider than the rods, which are cylindrical and thin. Cones are more than rods (fig. – 3b), this may be characterized by reptiles and birds of day activity [2], due to the protection of predators and the search for food because Iraqi spiny-tailed lizard lives in arid regions, the white-breasted kingfisher may have a high vision during the day and distinguishes its ability to predate and be consistent with [16, 17].

The outer limiting membrane is a colored and unclear light in some areas and the visual cell layer is separated from the external nuclear layer in both types, this layer consists of muller cells dendrites [6, 17].The external nuclear layer consists of the visual cell bodies, this layer difference in thickness in both species(table -1) and the number of rows of cells are between 1 – 2 in Iraqi spiny-tailed lizard (fig. – 3a), while the number of rows is between 3 – 4 in white-breasted kingfisher (fig. – 3b), this difference in thickness (table -1)

and the number of rows indicates the difference in visual cell density [5, 17], Iraqi spiny-tailed lizard and white-breasted kingfisher may depend on the bright vision in the search for food.

The external plexiform layer is difference in thickness (table -1), narrow and intertwines with the axons of the visual cells and the densities of both bipolar and horizontal cells in the studied species (fig. – 4a, 4b). The internal nuclear layer is characterized by its cells that are compact and diverse and consists of bipolar cells, horizontal cells and amacrine cells. This layer difference in thickness between studied species (table -1)and the number of rows ranges between 4 – 6 in Iraqi spiny-tailed lizard (fig. – 4a), this is different from the number in gecko[18] and the sleepy lizard *Tiliqua rugose* [19], while the number of rows in white-breasted kingfisher are between 8 – 16 (fig. – 4b), this is different from the number in *Falco tinnunculus* and *Streptopeliadecaocto*[17].

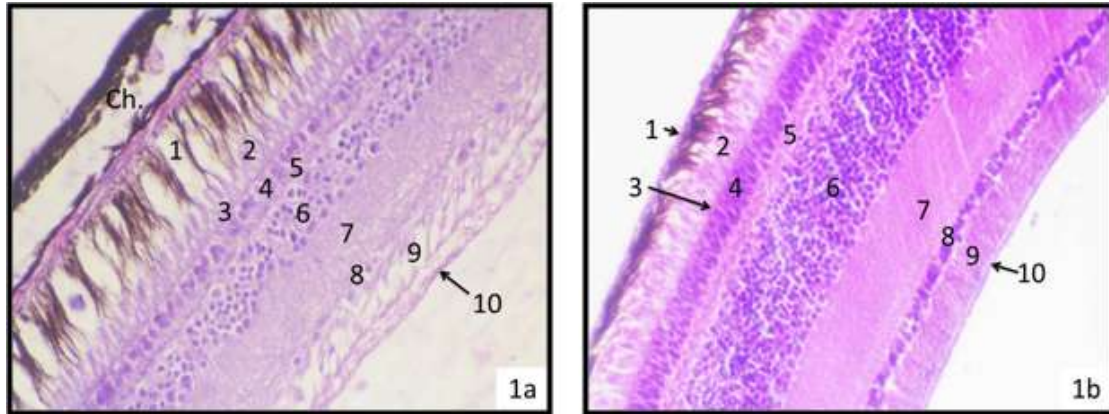
The internal plexiform layer is thicker than the external plexiform layer and consists of the Interlocking of the axes of both bipolar cells and amacrine cells, with dendrites of the ganglion cells that form single layer of the ganglion cell. Its layer cells are large in size and its nuclei are clear in both types (fig. – 5a, 5b). The thickness of the internal plexiform layer in both species (table -1), its depends on the density of the cells in the external and internal nuclear layers [16].The axons of the ganglion cells are collected to form the optic nerve fiber layer, which be thicker as it moves back towards the optic nerve that leaves the eye and reaches the brain. The optic nerve fiber layer is difference in thickness in both species (table -1). The inner limiting membrane separating the retina from the vitreous humor, it is a base plate of muller cells. The cells are giant that extended between the outer and inner limiting membrane and their nuclei are located within the internal nuclear layer in the studied species (fig. – 5a, 5b).These results consistent with [6, 17].

#### 4. Conclusion

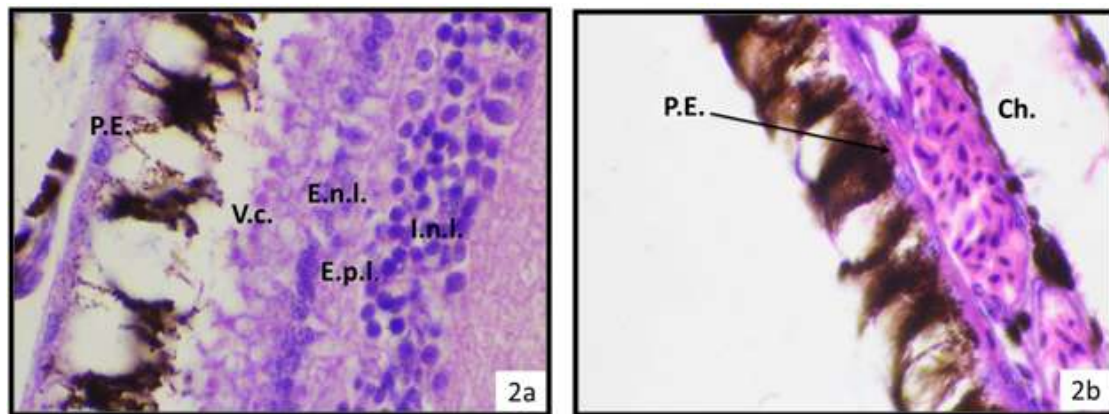
The difference in the density of the visual cells in both types is due to increased visual acuity and sensitivity to the light level. The proportion of cones is more than the rods in the studied species because it is a day activity and needs to absorb the largest amount of light to increase the visual acuity and color vision.

**Table 1:** Thicknesses of some neural retina layers in the two types

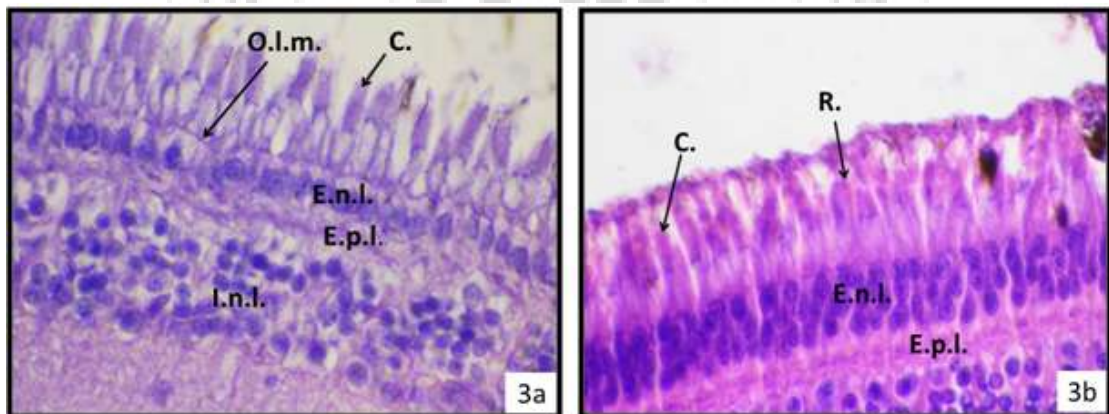
species	Neural retina layers (Mean ± SE)					
	ENL	EPL	INL	IPL	GCL	ONFL
<i>Saalaroricata</i>	80.00 ± 8.16	70.00 ± 23.80	320.00 ± 58.31	350.00 ± 49.32	70.00 ± 16.15	205.00 ± 58.52
<i>Halcyon smyrnensis</i>	155.00 ± 9.57	80.00 ± 8.16	525.00 ± 26.29	425.00 ± 2.17	105.00 ± 12.58	420.00 ± 82.46
LSD value	28.44 *	17.52 NS	45.36 *	39.11 *	27.53 *	64.91 *
* (P<0.05)						
(SE) Standard Error, (ENL) External Nuclear Layer, (EPL) External Plexiform Layer, (INL) Internal Nuclear Layer, (IPL) Internal Plexiform Layer, (GCL) Ganglion Cell Layer, (ONFL) Optic Nerve Fiber Layer						



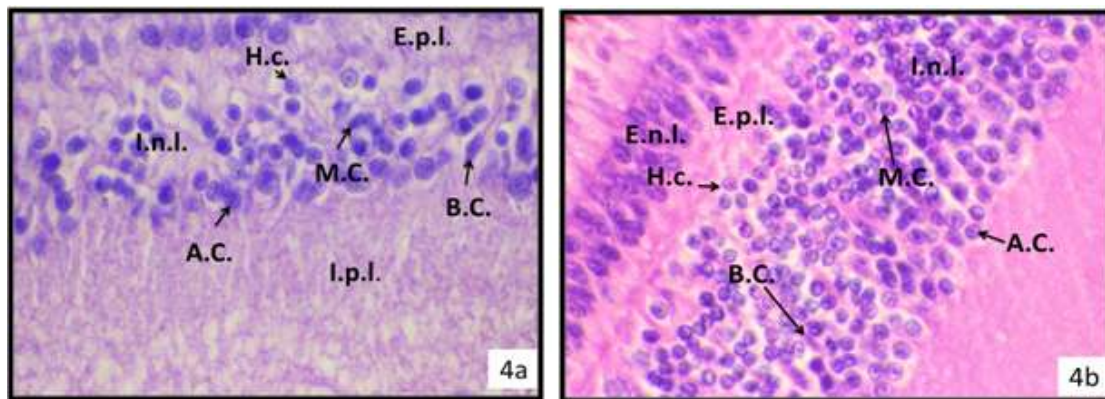
**Figure 1:** longitudinal section showing the layers of the retina in (a) Iraqi spiny-tailed lizard, (b) white-breasted kingfisher (400x (H&E Stain)). (Ch.) Choroid, (1) Pigmented epithelium, (2) Visual cell layer, (3) Outer limiting membrane, (4) External nuclear layer, (5) External plexiform layer, (6) Internal nuclear layer, (7) Internal plexiform layer, (8) Ganglion cell layer, (9) Optic nerve fiber layer, (10) Inner limiting membrane



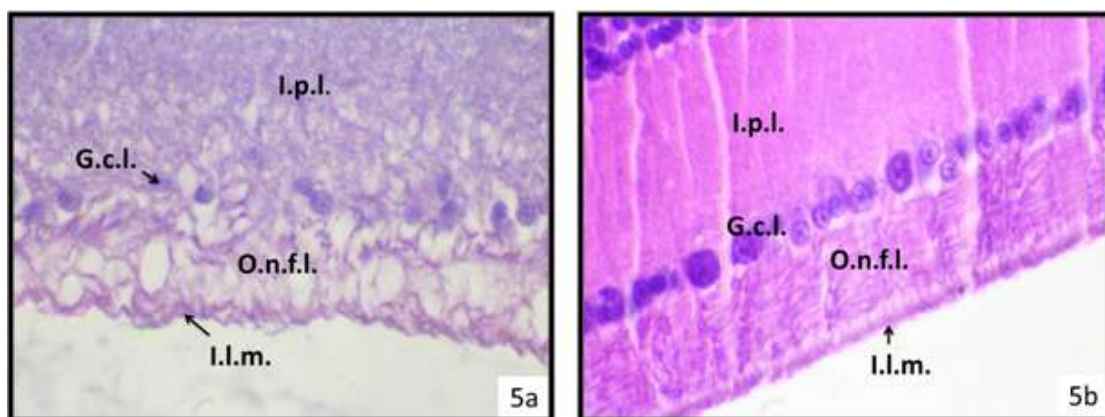
**Figure 2:** longitudinal section showing the layers of pigmented epithelium in the retina (a) Iraqi spiny-tailed lizard, (b) white-breasted kingfisher (1000x (H&E Stain)). (Ch.) Choroid, (P.E.) Pigmented Epithelium, (V.c.) Visual cell, (E.n.l.) External nuclear layer, (E.p.l.) External plexiform layer, (I.n.l.) Internal nuclear layer



**Figure 3:** longitudinal section showing the forms of the visual cells, the outer limiting membrane and the external nuclear layer in the retina (a) Iraqi spiny-tailed lizard, (b) white-breasted kingfisher (1000x (H&E Stain)). (E.n.l.) External nuclear layer, (R.) Rod, (C.) Cone, (O.l.m.) Outer limiting membrane, (E.p.l.) External plexiform layer, (I.n.l.) Internal nuclear layer



**Figure 4:** longitudinal section showing the external plexiform layer, cell shapes in the internal nuclear layer and the internal plexiform layer (a) Iraqi spiny-tailed lizard, (b) white-breasted kingfisher (1000x (H&E Stain)).(E.n.l.) External nuclear layer, (E.p.l.) External plexiform layer, (I.n.l.) Internal nuclear layer., (H.c.) Horizontal cell, (B.c.) Bipolar cell, (A.c.) Amacrine cell, (M.c.) Muller's cell, (I.p.l.) Internal plexiform layer



**Figure 5:** longitudinal section showing the internal plexiform layer, the ganglion cell layer, the optic nerve fiber layer and the inner limiting membrane in the retina (a) Iraqi spiny-tailed lizard, (b) white-breasted kingfisher (1000x (H&E Stain)).(I.p.l.) Internal plexiform layer, (G.c.l.) Ganglion cells layer, (O.n.f.) Optic nerve fiber layer, (I.l.m) Inner limiting membrane

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