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Histological Adaptations of the Retina in the Black Francolin (*Francolinus francolinus*, Linnaus, 1766) and the Caucasian Squirrel (*Sciurus anomalus*, Gmelin, 1778)

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Abstract
Ecological diversity between the diurnal bird of *F. francolinus* and the diurnal squirrel of *S. anomalus* reflects the ability to adapt to their vision environments. The histological characteristics of the retina in *F. francolinus* and *S. anomalus* were studied using light microscopy. The retina of *F. francolinus* is avascular, while that of *S. anomalus* is vascular. The retina in both species consists of ten layers. In both species, the retina includes cones and rods, and the increased cone density is adapted for daytime and color vision. The retina of *F. francolinus* contains a shallow fovea for visual acuity. This shallow fovea was characterized by the presence of all retinal layers in the pit that provided a monocular view for near-distance sight. The *S. anomalus* retina was distinguished by the presence of blood vessels extending within the ganglion cell and nerve fiber layers. Thus, visual acuity played an important role in the environment of both *F. francolinus* and *S. anomalus* as well as in their behaviors.

Keywords: cones, *Francolinus francolinus*, retina, rods, *Sciurus anomalus*, shallow fovea

التكيفات النسجية لشبكية العين في الدراج الاسود (*Francolinus francolinus*, Linnaus, 1766) والسنجاب القوقازي (*Sciurus anomalus*, Gmelin, 1778)

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الخلاصة
يعكس التنوع البيئي بين الطائر النهاري *F. francolinus* والسنجاب النهاري *S. anomalus* قدرتهما على التكيف البصري مع بيئتهما. تمت دراسة خصائص النسجية لشبكية العين في *F. francolinus* و *S. anomalus* باستخدام المجهر الضوئي. شبكية العين في *F. francolinus* لاوعائية، في حين أن شبكية *S. anomalus* وعائية. تتكون الشبكية في كلا النوعين من عشر طبقات. تحتوي الخلايا البصرية في الشبكية في كلا النوعين من المخاريط والقضبان وتزداد كثافة المخاريط لاجل التكيف مع الرؤية النهارية

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والألوان. تحتوي شبكية العين في *F. francolinus* على حفرة ضحلة لاجل دقة الرؤية. تميزت هذه الحفرة الضحلة بوجود جميع طبقات الشبكية في النقرة لتوفير رؤية أحادية للمدى القريب. تميزت شبكية عين *S. anomalus* بوجود اوعية دموية تمتد داخل طبقات الخلايا العقدية والألياف العصبية. وهكذا، تؤدي حدة البصر دورًا مهمًا في بيئة كل من *F. francolinus* و *S. anomalus* وكذلك سلوكها الحيوي.

1. Introduction

Vision has a close connection between functional characters and species-particular ecology [1]. Eyes are photosensitive organs that interpret the form, intensity, and colour of light reflected from objects. The eyeball is located within the orbits of the skull and consists externally of tunica fibrosa that maintains an eye's overall shape [2]. Internally, the eye contains the retina that carries sight information from the external environment to the cerebrum [3]. The retina is composed of the pigment epithelium and the neural retina, which contains visual cell layers that rods and cones in varying numbers between most diurnal and nocturnal vertebrates [4, 5]. The central retina has a high concentration of cones called the fovea for high-acuity vision [6]. Birds have one or two foveae: deep fovea and shallow fovea [7]. The variations of acute sight indicate the adaptations for species required to overcome environmental challenges [8].

This study aimed to determine the extent of retinal tissue variation in two distinct Iraqi wild species, notably class, environment, and feeding habits. The black francolin (*Francolinus francolinus*, Linnaus, 1766) belongs to class Aves and is omnivorous. It lives in lowlands, valleys, reed plains, and agricultural lands in Iraq [9]. The caucasian squirrel (*Sciurus anomalus*, Gmelin, 1778) belongs to the class Mammalia and is herbivorous. This squirrel inhabits forests in the mountains and valleys of northern Iraq [10].

2. Materials and Methods

For this study, three adults of each species (two males and one female of *F. francolinus* and three males of *S. anomalus*) were procured from the Al-Ghazel market in Baghdad. The specimens were euthanized by chloroform, and their eyes were removed. The eyeballs were immersed in a fixative (Bouin's solution) for ten h., washed with 70% ethanol, and then stored in the same solution [11]. The next day, the samples were routinely processed using the wax paraffin technique. Subsequently, the eye tissues were stained using Harris's-Haematoxylin & Alcoholic-Eosin (H&E) [12] and Masson-Trichrome (MT) [13]. The retina tissues were observed under a Meiji compound microscope, and a Canon camera was used to take photomicrographs.

3. Results

3.1 The retina of the black francolin

The results showed that the retina of *F. francolinus* was avascular and composed of ten distinct layers (Figure 1A). The first layer, pigmented epithelium of *F. francolinus* consists of a single layer of cuboidal cells containing oval-shaped nuclei. These epithelial cells contain melanin granules and extend abundant cytoplasmic processes that enclose the visual cells outer segments (Figure 1B). The second layer, the visual cells, contains rods, single cones, and double cones. These cells were formed by the outer and inner segments and differentiated by characteristics of their outer segments of cones, which are conical in shape and wide, while rods were revealed to be taller and cylindrical. The layer was observed to have more cones relative to rods. The third layer is a lightly stained region located between the photoreceptor layer and the outer nuclear layer. It is known as the external limiting membrane. The fourth layer, the outer nuclear layer, contains cell bodies of both rods and cones, and their nuclei are organized in 2–4 rows, a distribution that varies in the retina. The fifth layer, the outer plexiform layer, was composed of visual cell axons that connect to the dendrites of bipolar and horizontal cells (Figure 2A and B).

The sixth layer, the inner nuclear, includes different types of neurons, especially bipolar neurons, horizontal cells, amacrine cells, and Müller cells, as well as a row of nuclei arranged between 5 and 14. The seventh layer, the inner plexiform layer, contains bipolar and amacrine cell axons that connect with the ganglion cell processes. The eighth layer consists of ganglion cells arranged in one to three distinct rows. The axons of these ganglion cells form the ninth layer, known as the nerve fiber layer, which converges at the optic nerve head to form the optic nerve. The tenth layer is the internal limiting membrane created by the terminal processing of Müller cells that separate the tunica interna from the vitreous body (Figure 3A and B).The retina includes the shallow fovea shallower shape and layers of the retina in the pit. In addition, the internal retina layer cells are less than in the region near the parafovea (Figure 4A and B).

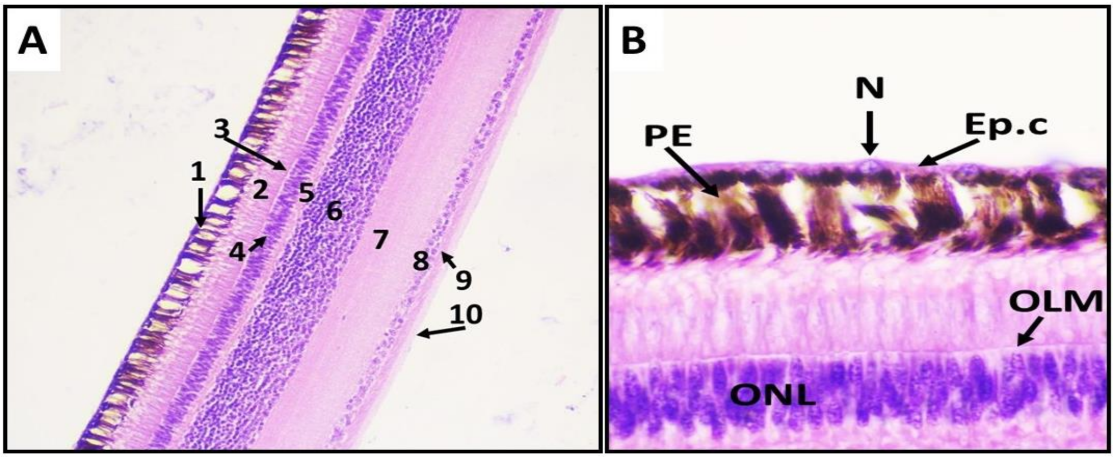

Figure 1: Cross section of the retina in *F. francolinus* illustrates A: all retina layers; B: structure of pigment epithelium. (1) Pigmented Epithelium (PE), (2) visual cells layer, (3) Outer Limiting Membrane (OLM), (4) Outer Nuclear Layer (ONL), (5) outer plexiform layer, (6) inner nuclear layer, (7) inner plexiform layer, (8) ganglion cell layer, (9) nerve fiber layer, (10) inner limiting membrane, Nucleus (N), Epithelial cell (Ep.c). [A:100X (H & E stain); B:1000X (MT stain)].

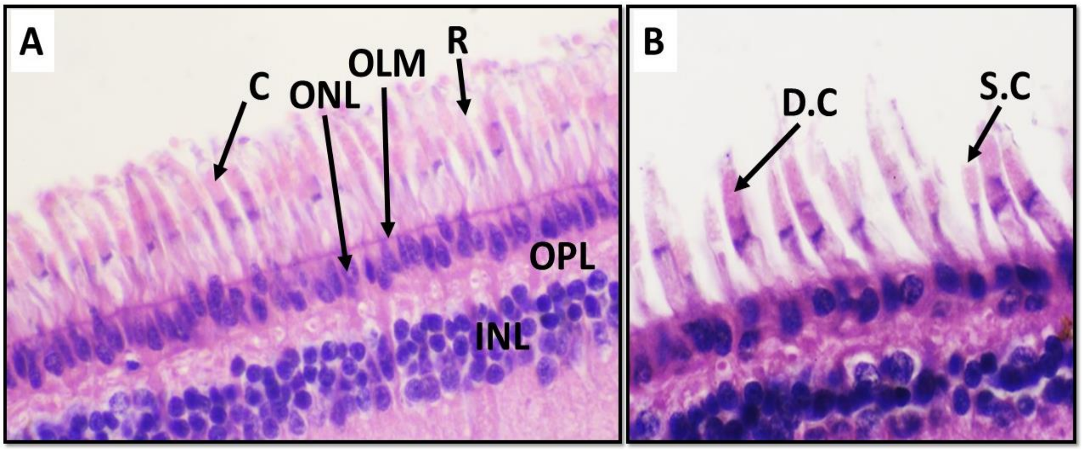

Figure 2: Cross section of the retina in *F. francolinus* showing the shapes of visual cells, Outer Limiting Membrane (OLM), Outer Nuclear Layer (ONL),Outer Plexiform Layer (OPL), Inner Nuclear Layer (INL), Cone (C.), Rod (R.), Double Cone (D.C), and Single Cone (S.C) [1000X, A:(MT stain) & B: (H&E stain)].

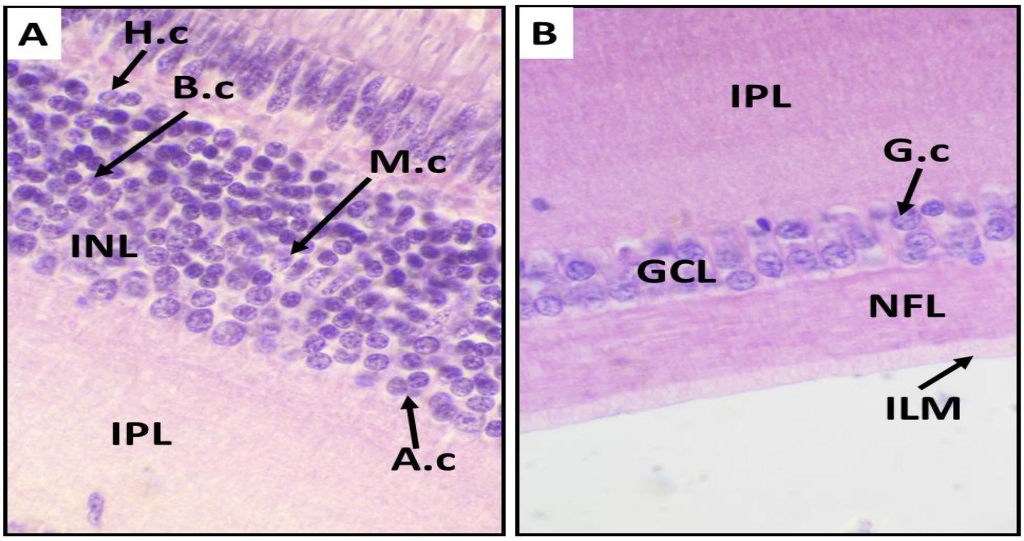

Figure 3: Cross section of the retina in *F. francolinus* illustrates A: nuclei of cells in the Inner Nuclear Layer (INL), notably Bipolar cell (B.c), Horizontal cell (H.c), Muller cell (M.c), Amacrine cell (A.c); B: Inner Plexiform Layer (IPL), Ganglion Cell Layer (GCL), Ganglion cell (G.c), Nerve Fiber Layer (NFL), Inner Limiting Membrane (ILM) [1000X A:(MT stain)& B:(H&E stain)].

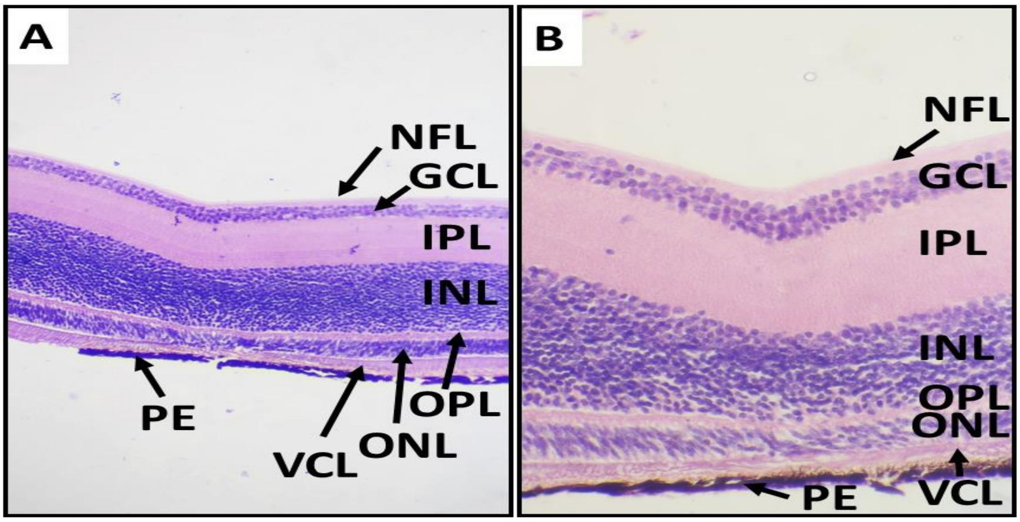

Figure 4: Cross section of the retina showing the structure of shallow fovea in *F. francolinus*. Pigmented Epithelium (PE), Visual Cell Layer (VCL), Outer Nuclear Layer (ONL), Outer Plexiform Layer (OPL), Inner Nuclear Layer (INL), Inner Plexiform Layer (IPL), Ganglion Cell Layer (GCL), and Nerve Fiber Layer (NFL) [A:100X (H&E stain); B: 400X (MTstain)].

3.2 The retina of the caucasian squirrel

Histologically, the retina of *S. anomalus* was characterized by being vascular and composed of ten distinct layers. The retinal pigment epithelial consists of a monolayer of cuboidal epithelial cells containing pigment granules in the cytoplasm, and the terminal processes of these cells extend toward the outer segments of the photoreceptors (Figure 5A and B). The layer of retinal visual cells includes both rods and cones, which exhibit distinct outer and inner segment structures. The outer segments are cylindrical in rods and funnel-shaped in cones, and this layer contains a significantly higher proportion of cones compared to rods. The outer limiting membrane of the retina was located between the inner segments of visual cells and the outer nuclear layer. The soma of these cells contains the nuclei of both rods and cones within the outer nuclear layer of the retina that is arranged in 2-3 rows. The retinal outer plexiform layer was formed by connections of visual cells axons and the extensions of bipolar cells and horizontal cells (Figure 6A and B).

The layer of the retinal inner nuclear includes the somas of bipolar, horizontal, amacrine, and Müller cells, and includes 6-12 rows of nuclei. The retinal inner plexiform layer is formed by interlaced processes of the ganglion cells and cells of the inner nuclear layer. The retinal ganglion cell layer consists of single-layered ganglion cells, and is usually arranged in 1-2 rows. The retinal nerve fiber layer is created by axons of ganglion cells. The nerve fibers meet to create the optic nerve in the central retina. The retinal inner limiting membrane was located at the boundary of the retina between Müller cells termination and the vitreous body. The retina was also characterized by the presence of blood vessels located between the ganglion cell layer and nerve fiber layer (Figure 7A and B).

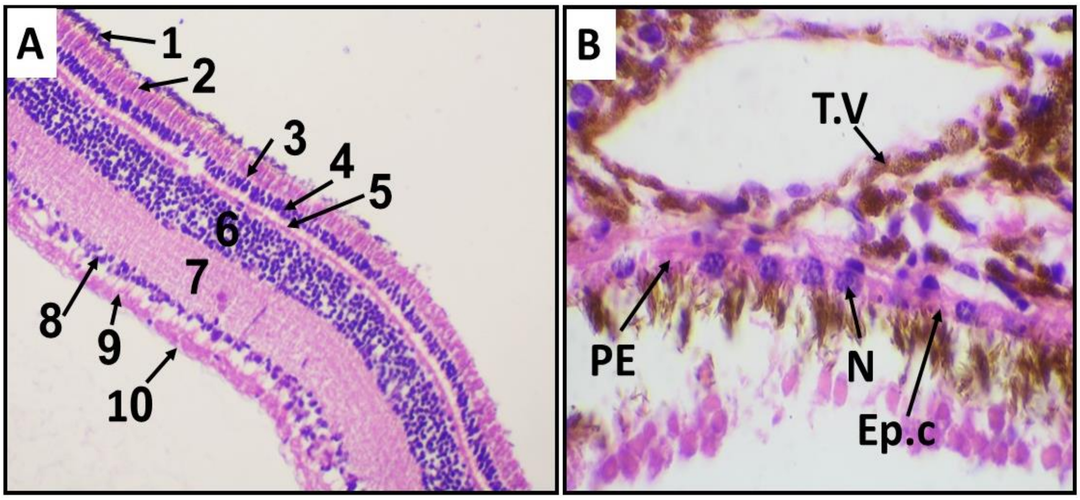

Figure 5: Cross section of the retina in the *Sciurus anomalus* illustrates A: all retina layers; B: structure of pigment epithelium. (1) Pigmented Epithelium (PE), (2) Visual cells layer, (3) External limiting membrane, (4) Outer nuclear layer, (5) Outer plexiform layer, (6) Inner nuclear layer, (7) Inner plexiform layer, (8) ganglion cell layer, (9) Nerve fiber layer, (10) internal limiting membrane, Nucleus (N), Epithelial cell (Ep.c), Tunica Vasculosa (T.V). [A:200X (H&Estain); B:1000X (MT stain)].

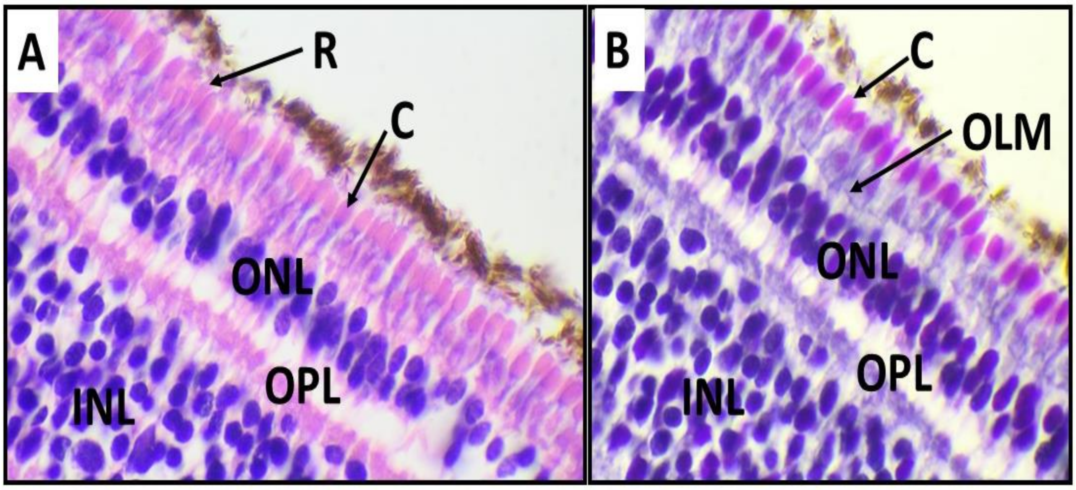

Figure 6: Cross section of the retina in the *Sciurus anomalus* showing the shapes of visual cells, Outer Limiting Membrane (OLM), Outer Nuclear Layer (ONL), Outer Plexiform Layer (OPL),Inner Nuclear Layer (INL), Cone (C.), and Rod (R.) [1000X, A:(H&E stain) & B:(MT stain)].

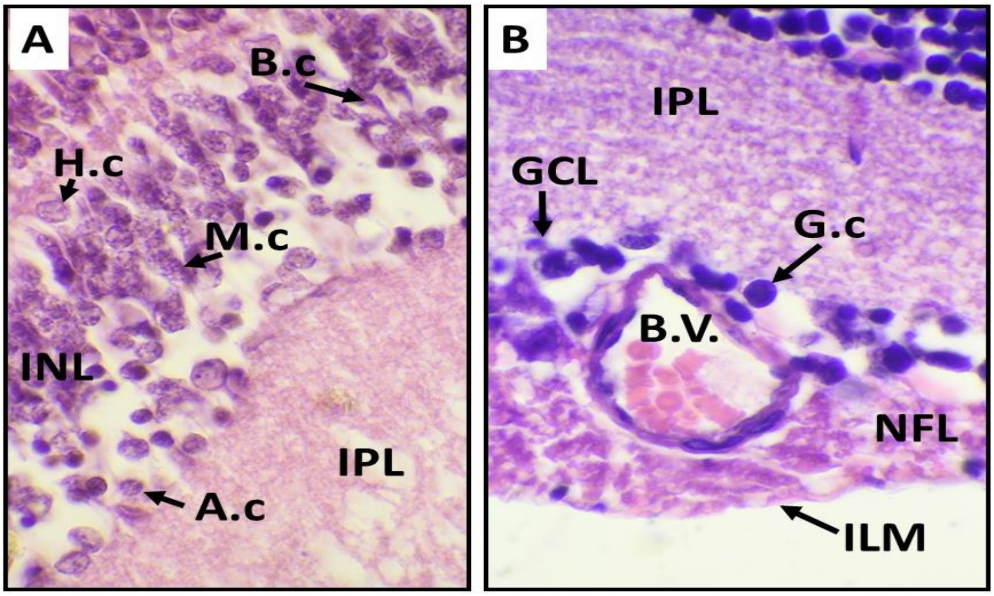

Figure 7: Cross section of the retina in *Sciurus anomalus* illustrates A: the Inner Nuclear Layer (INL) contain cell shapes of Bipolar cell (B.c), Amacrine cell (A.c), Horizontal cell (H.c), Muller cell (M.c); B: Inner Plexiform Layer (IPL), Ganglion Cell Layer (GCL), Ganglion cell (G.c), Nerve Fiber Layer (NFL), Blood Vessel (B.V.), and Inner Limiting Membrane (ILM) [1000X A:(MT stain) & B:(H&E stain)].

4. Discussion

The retinal structure of *F. francolinus* and *S. anomalus* is generally similar to other vertebrates. All retinal layers are distributed similarly in vertebrates, with the pigmented epithelium at the beginning and the inner limiting membrane at the end [14, 15, 16]. However, variations occur due to the visual adaptation to diverse environments. The retina is responsible for the accurate transfer of information from external light to the cerebrum, where neural signals are converted into eyesight [3, 17]. The retinal pigment epithelium of *F. francolinus* and *S. anomalus* is identical to that observed in other vertebrates. The arrangement of melanosomes at the base of the cells in this layer is essential for the vision of diurnal vertebrates. This layer is important for phagocytosis of debris from cones and rods outer segments, the selective transmission of materials to photoreceptors, light absorption, metabolizing vitamin A precursors in the visual pigments, and defending against photooxidation [18, 19, 20].

In general, the photoreceptor layer exhibits increased cone density compared to rods that are well adapted to daylight conditions. Cones play a role in color eyesight in bright light, while rods are in dim light [21]. The cones predominate in diurnal black francolins, which may enable the retina to adapt to a wide variety of light levels for color sight in order to seek food and protect against predators. The increase of cone-type visual cells in the *S. anomalus* retina enhances the ability to see colors and daytime vision. These cells may be important for squirrel's daily behavior, which includes moving between trees and searching for food. The retina of *E. caeruleus* and *L. michahellis* have an abundance of cones that enable color vision and high visual acuity to search for food without moving the eyes [22, 23]. In vertebrates, the retinal outer limiting membrane is generally comparable in structure to that of many of the class's species. It is believed that the outer limiting membrane contributes to the mechanical strength that keeps the retina's structure intact and protects the retina against macromolecules [3, 23]. The various numbers of the external nuclear layer rows between *F. francolinus* and *S. anomalus* revealed a variation in the photoreceptor density. The presence of various visual cell somas in the external nuclear layer for acute vision of diurnal animals [1, 8]. The outer plexiform layer density varies through the retina of *F. francolinus* and *S. anomalus*. This variation suggests a higher density of bipolar and visual cells in this layer for acute sight [8, 15]. The current study showed that the retinal inner nuclear layer of *F. francolinus* and *S. anomalus* has a variety of rows that may be because of various neuron patterns. The layer of inner nuclear contains different rows that lead to more efficient visual signals in regions with high vision [8]. The retinal inner plexiform layer of *F. francolinus* and *S. anomalus* is wider than the layer of outer plexiform; that may be because of the highly dense synaptic interaction of the neurons. This may reflect the adaptation of the retina that responds to changing light levels in varying environments [8, 24]. The ganglion cell numbers vary between *F. francolinus* and *S. anomalus*. These cells receive visual input from bipolar and amacrine cells. The axons of retinal ganglion cells create a nerve fiber layer to transport visual information to the brain [23]. The Müller cells create the internal limiting membrane, which serves as the structural barrier between the inner retina and the vitreous body. The Müller cells preserve cell viability and homeostasis [25].

The retina of *F. francolinus* has a shallow fovea, which contains a density of cones and a varying number of ganglion cell rows. Perhaps this fovea allows monocular vision in bright light to focus on the things for capturing seeds and small invertebrates. Other birds have different foveal depths based on how the layers of the internal retina in the fovea pit are arranged to capture small or quick-moving things [7, 26]. The Müller cell can assist in magnifying the picture of the fovea center and focusing light into an area inside and surrounding the center of the fovea [27, 28]. The retina of *S. anomalus* is distinguished by the presence of blood vessels between the ganglion cell layer and nerve fiber layer, supplying oxygen and nutrition to the inner retina layers.

5. Conclusion

The structural adaptations observed in the retina of *F. francolinus* and *S. anomalus* connect the visual system with the surrounding environment. The arrangement of the density of cone-type visual cells reflects an optimization for daylight vision in high illumination. The shallow fovea of *F. francolinus* provides visual acuity and color vision when seeking meals. The presence of blood vessels reflects the adaptation of the *S. anomalus* retina to suit the diurnal activity of this squirrel.

6. Ethical responsibilities of authors

This experiment involving samples was conducted in accordance with guidelines of the animal ethics committee under approval number (861; 8/ 10/2024) of the University of Baghdad, College of Science for Women, Department of Biology, Baghdad, Iraq.

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Conflict of interest

Authors declare that they have no conflicts of interest.

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