International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **4(11),** 43-54, November **(2015)** *Int. Res. J. Biological Sci.*

The Development of Ophthalmic Apparatus of Malpolon Monsspesullanus (Squamata-Serpentes) in Postovopositional Stages

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Available online at: www.isca.in, www.isca.me
Received 21st October 2015, revised 29th October 2015, accepted 9th November 2015

Abstract

The present investigation aimed to give a special attention on an ophthalmic apparatus development of a species of mildly venomous rear-fanded Colubrids snake; the Montpellier snake. The development of the eye was studied in different developmental stages; 1cm; 1.2cm, 1.5cm, 1.9cm, 4cm, 5.0cm, 5.5cm, 6.5cm, 7cm and 8cm total body length. The eye starts its growth as a bilateral evagination from the floor of the diencephalon. Latter, this evagination will form the different components of the eye (retina, iris, ciliary proceses, conus papillaris, lens, cornea, choroid, and sclera). Moreover, the innervations of ophthalmic apparatus appear in the early stage of development as an optic stalk and the latter develops until reach the fully formed optic nerve.

Keywords: Malpolon monsspesulanus, squamata, eye development.

Introduction

The greater part of the work dealing with eye and its development (apart from that on the human eye) has been done and published on fishes, amphibians and birds. So far as we have been able to ascertain limited investigations have been made on reptilian eye's of any kind specially snakes.

The variations of eyes are extremely important to the survival and reproduction of individual vertebrates. In the Ophidia, whose eyes are generally relatively large and well-developed, and in which vision is thinking to be one of the main senses[1]. Moreover, the eyesight of snakes is quite useless and the eye is reduced or absent in some fossorial snakes. Seriously, they depend more on the other special senses; the subordination of vision to olfaction and vibration[2].

The eye morphology across the embryological stages of *Sphaerodactylus argus show* significant changes[3]. The development of eye was recorded in nine species of 88 embryos. In different sphaerodactyl species, eye development is examined[4]. In addition, a unique embryonic developmental pattern of the pigmented epithelium of retinal in dwarf geckos was recorded, and comparing it with embryonic information of different lizard families[4]. Measuring of the structures in the anterior segment of the eye of five different snake species using ultrasound imaging can serve as orientative values for snakes examined for ocular diseases[5]. Morphological studies of squamate fossorial reptiles's eye are reported[6]. The submicroscopical structures peculiarities in the cornea are investigated in some reptilies (grass-snake, lizard, turtle) which live in different habitats[7]**.** The early article was recorded, on the optic system in vertebrates, which included observations on Natrix[8]. Retinal light sensitive cells (photoreceptors) and its pigment of typical boid snake (*Python regius*) were characterized using microspectrophotometry and scanning electron microscopy[9]. Also, other boid retinas are examined with using light microscope[10]. Morphological studies on the layer of retinal in the sea-snake (*Pelamis platurus)* were investigated using Silver stained and Golgi-Cox preparations for light microscopy in addition, Electron microscopy for thick epoxy-embedded specimens[11]. Comparative morphology on the spectacle of the snake utilizing light, transmission electron microscope was carried out[12.]

Material and Methods

Sample collection: *Malpolon* (Coelopeltis) *monspessulanus* (Family: Colubridae) which includes the common living snakes[13]. Numbers of pregnant females were collected by field work during May and June. At the end of June, the pregnant females laid their eggs freely on the sand. The eggs are whitish yellow and elliptical in its shape. Where, eggs were incubated in moist soil at room temperature. The average period of incubation is 63 day. Thus, we can collect set of embryos at different stages of *Malpolon monsspesulanus.*

Morphometeric estimation and preparation of permanent histological slides: The embryos were removed from their shells carefully, and only those which were living were euthanized according to the standard guidelines of The Institutional Animal Care and Use Committee (IACUC) for ectothermic animal. Healthy ones were added in Bouin's solution for fixative (24-48 hours) according to the size of embryos are used. In Cairo during the breeding time of *Malpolon monsspesulanus,* the room temperature range between 32–35° during the day, but at the night, it could be considerably cooler. In the wild, diurnal temperature fluctuations occur, but

the female snake can influence this by choice of breeding site (depth and cover). The two factors (incubation temperature and fluctuations in temperatures) effect on embryos development[14]. Therefore, measurements of total body length are used, and authors are driven to adopting the total body length as their standard of comparison. The subsequent successive embryos of *Malpolon* were noted: stage-1 (Total body length: 1.0 cm); stage-2 (total body length: 1.2cm); stage-3 (total body length: 1.5cm); stage 4 (Total body length: 1.9cm); stage 5 (Total body length: 4cm); stage 6 (Total body length: 5.0cm); stage-7 (Total body length: 5.5cm); stage 8 (Total body length: 6.5cm); stage 9 (Total body length: 7cm); stage 10 (total body length: 8cm).

Embryos were treated with ascending series of ethyl alcohol and then cleared with xylene. Thereafter, the specimens were embedded in a paraffin wax. This was followed by sectioning to head of embryos transversely at 10 microns thickness using Reichert microtome.

The sections of each specimen were mounted serially on microscopic slides and set for staining. The latter was carried out by haematoxylin (Ehrlich) and counterstained by eosin to obtain permanent histological preparations.

A photomicrograph using Zeiss photomicroscope: The visual apparatus is examined in these sections. Micrographs of several stained sections were captured using Zeiss photomicroscope supplied by Canon digital camera to describe the different developmental changes of the eye and its relation to the different neighboring structures.

This one of series of papers, which was studied the development of sense organs in *Malpolon monsspesulaus* as an important means for adaption of the surrounding environments. The work has been carried out in the Department of Zoology (Comparative Anatomy and Embryology Lab), Faculty of Science, and Cairo University.

Results and Discussion

Stage-1 (total body length: 1.0cm): The eye (figure-1) starts its development as a bilateral vesicular evagination,(the primary optic vesicle, OP.V), from the ventral part of the lateral side of the diencephalon (DIC). The wall the vesicle is formed from more or less rounded neuroblasts. By microscopical investigation to the transverse serial sections reveals that, the optic vesicle has a large cavity called the optocoele (OPC). This cavity is connected with the cavity of the diencephalon through a large cavity of the future the hollow optic stalk (OP.ST). In this stage, the ectoderm opposite to the protruded primary optic vesicle becomes thick. This thickening ectoderm represents the primordium lens placode (figure-1, L.P.PR).

Stage 2 (Total body length: 1.2cm): As the development proceeded in this stage, the lens placode primordium increases in its thickness forming lens placode (figure-2, L.P). In some specimens of the same total body length, shortly after the lens placode is formed, it shows an invagination towards the optic vesicle forming lens pit (figure-3, L.PI). The lens opens to the exterior by a minute opening. The outer wall of the primary optic vesicle invaginates to inwards forming a double wall structure; the optic cup; the secondary optic vesicle (figures-2, 3, OP.CU). This vesicle is still associated with the diencephalon by a hollow optic stalk (OP.ST). The cavity of the primary optic vesicle, optocoele (OPC) becomes reduced, as a result of invagination. A new cavity, the future viterous space (secondary optic vesicle), is between the lens placode (L.P) laterally and the optic cup medially. (Figure-2, VTS).

Stage 3 (Total body length: 1.5cm): In this stage (figure-4), the double layered optic cup (the secondary optic vesicle) is formed. The secondary optic vesicle attached to the diencephalon through a solid optic stalk (OP.ST). The cavity of the primary optic vesicle, optocoele (OPC) becomes more reduced than previous stage, as a result of invagination. A well developed viterous space appears between the newly formed lens vesicle (figure-5, L.V) laterally and the optic cup medially (figsure-4, 5, VTS). The lens vesicle gradually falls down into the optic cup. This vesicle attains round shape with a narrow cavity (figure-5).

Stage 4 (total body length: 1.9cm): In the current study , the wall of the optic cup is incomplete due to presence of a break or cleft in its ventral lip, this break forms the retinal (choroidal) fissure (figures-6, 7. RT.FS). the latter allows to the mesenchymal cells and blood vessels enter through the viterous cavity. In this stage, a double layered structure (the optic cup, OP.CU), or the secondary optic vesicle was formed from future outer thin pigmented layer of the retina; PG.LA and future inner thick neural layer of the retina; NE.LA (figure-7). In addition, the future viterous space becomes large. In this stage, an oval lens vesicle (figure-8) becomes larger as compared with previous stage. The medial wall of the lens vesicle becomes slightly thicker than its lateral one. The distal surface of the medial wall of the lens vesicle projects into its cavity to attain a cresentic shape. The cells of the medial wall of the lens vesicle elongate. The nuclei of the latter rearranged to occupy a midway zone between its two surfaces. The region which contains the nuclei is termed lenticular portion of the lens (LE.PO). Meanwhile, the lateral portion becomes thin forming the future lens epithelium (L.EP). The lens epithelium and corneal epithelium (CR.EP) become completely separated by a cavity, which is the anterior eye chamber (AEC). In this stage, the ectoderm lies opposite to the lateral wall of the lens vesicle forms the future corneal epithelium (figure-8, CR.EP). The latter, formed from a single row of squamous and a double rows of cuboidal epithelia.

Stage 5 (total body length: 4cm): In this growing stage (figure-9), the lens is rounded. Its body increases in thickness, where the cells of the medial wall, the lenticular portion (LE.PO) continue to elongate and become more fibrous. Meanwhile, the

lateral wall of the lens formed from a thin uniform epithelium (L.EP). As a result, there is a reduction in the lens cavity. The medial surface of the lens becomes surrounded by a thin layer of fibres which forms the lens capsule (L.CA). The fibrous material that connects the lens and the optic cup represents the lens ligament (L.LT).

Stage 6 (Total body length: 5cm): Here, the cells of the outer pigmented layer (PG.LA) of retina begin to form pigmented granules, while those of the inner neural layer (NE.LA) become considerably thicker (figure-10). Inspection of the ganglionic layer (GA.LA) of the inner neural layer reveals that its cells start forming nerve fibres, which converge towards the optic stalk forming the optic nerve primordium (figures-10,11,12, OP.N.PM).

The latter extend posteromedially to form the optic chiasma primordium (figure-12, OP.CH. PM) then enter the ventral surface of the mesencephalon. In the current stage, the closure of the retinal (choroidal) fissure (figure-11, RT.FS) begins at the posterior end of the developing eye. This closure is synchronized with the formation of the elongated cauda of the optic nerve and with the simultaneous formation of atrophied conus papillaris (CO.PM). There is a fold of the inner retinal layer extending slightly into the lumen of the optic stalk. Fusion begins at the apex of the fold through active cellular proliferation. The fold then bridges the lumen of the optic stalk providing a pathway for the exit of the nerve fibres from the retina. A moderate number of fibres are seen leaving the eye by this route. The conus papillaris primordium (figure-10, CO.PM) projects into the vitreous humor as a low ridge along the line of fusion of the retinal fissure. The latter, is still persisted at the anterior end of the eye. Through the fissure pass mesodermal elements and blood vessels into the developing viterous humor (figure-11).

The lens vesicle of this stage (figure-13) is still rounded in shape and surrounded with fibrous lens capsule (L.CA). The lens epithelium (L.EP) becomes thinner than in the previous developmental stage. The cells of the lenticular portion of the medial wall continue their elongation and become more fibrous. Here, is a slight extension of the edges of the layers of the optic cup external to the lens vesicle. Outer of this extension there is an aggregation of mesenchymal cells and vascular elements. These extensions together with the mesenchymal aggregates form the primordium of iris and ciliary process (figure-13, IR.PM). In the developing stage, the cornea primordium (figures-13,14, CR.PM) appears formed of an outer epithelium followed by a fibrous membrane and single mesenchymal cell layer.

The mesenchymal cells migrate in, from all sides along the inner surface of fibrous membrane. In the current stage, a little amount of blood capillaries appear around pigmented layer of the retina representing choroid primordium (figure-14, CD.PM). By light microscope examination in the same total body length, the optic nerve primordium becomes invaded by fibres from gangalonic layer of retina forming an optic nerve (figure-14, OP.N).

Stage 7 (total body length: 5.5cm): The optic chiasma (figure-15, OP.CH) becomes more developed than in the previous stage, enter the ventral surface of the mesencephalon.

The lens epithelium (L.EP) of the lens vesicle becomes thinner than previous stage. The cells of the lenticular portion (LE.PO) of the lens become crystalline in its appearance (figure-16).

Stage 8 (total body length: 6.5cm): In this stage (figure-17), the inner neural (sensory) layer of the retina becomes thicker. It differentiates into an outer layer of photoreceptor (PH.LA), a middle thick layer of bipolar neurons (BN.LA) and an inner ganglionic layer (GA.LA).

Here, as the growth proceeds the nerve fibres have developed from the cells of the ganglionic layer of the inner neural layer of the retina forming a stout optic nerve. This nerve forms a well developed optic chiasma than in the previous stage (figure-18). In this stage, the outer pigmented layer (figure-19, PG.LA) of the retina consists of two layers of cuboidal cells that shows a relatively high degree of pigmentation. The first appearance of the sclera primordium (figures-19, SC.PM) of *Malpolon* takes place during this period of development as a weakly mesenchymal ring around the optic vesicle.

Stage 9 (total body length: 7cm): In this stage (figure-20), the different layers of the retina appear clear at the retinal axis than at its margin. The neural retina becomes more developed than the previous stage. It consists of six layers which are from outside to inside: a photoreceptor layer (PH.LA), the outer reticular layer (O.R.LA), a bipolar neural layer (BN.LA), an inner reticular layer (I.R.LA), a ganglionic layer (GA.LA) and a nerve fiber layer. In *Malpolon*, the fiber of the growing optic nerve decussate directly forms a growing optic chiasma (figure-21). The sclera (figure-20, SC) in this stage become more fibrous. Regarding, the developing iris, an extension of the edges of the layers of the optic vesicle (inner (neural) retinal, pigmented layer) external to the lens vesicle. Also, the inner part of the mesenchymal aggregates grows in correlation with the retinal margin forming the developing iris (figure-22, IR). The developing cornea (figure-23) becomes thicker and consists of four layers. The outer most layer; the corneal epithelium (CR.EP), is formed of one cuboidal cell rows and a single one of squamous epithelium. The corneal epithelium rests on a thin layer of compressed collagenous fibres representing Bowman′s membrane primordium (BO.MM). This is followed by a layer of mesenchymal connective tissue (MC.CT); it is the thickest layer formed only of connective tissue. The inner most layer is the corneal endothelium (CR.EN), it consists of an irregular single row of squamous epithelial cells.

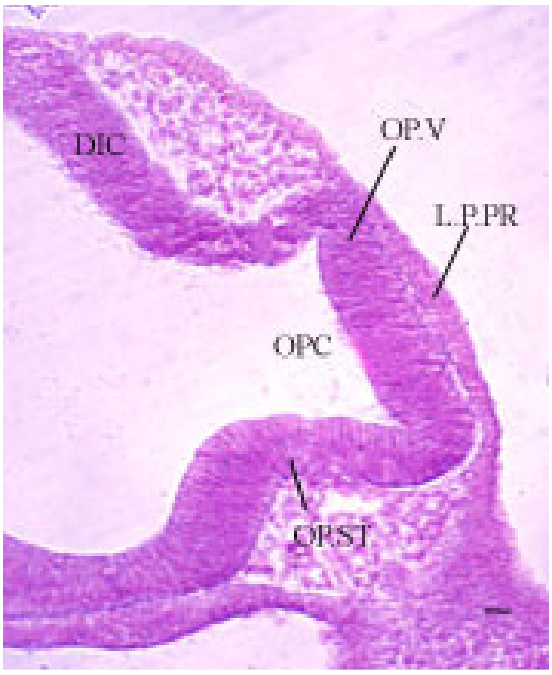

Figure-1
At 1 cm total body length, showing well organized diencephalon (DIC), lens placode primordium (L.P.PR), optic vesicle (OP.V), optic stalk (OP.ST), optocoele (OPC). Scale bars,25µm

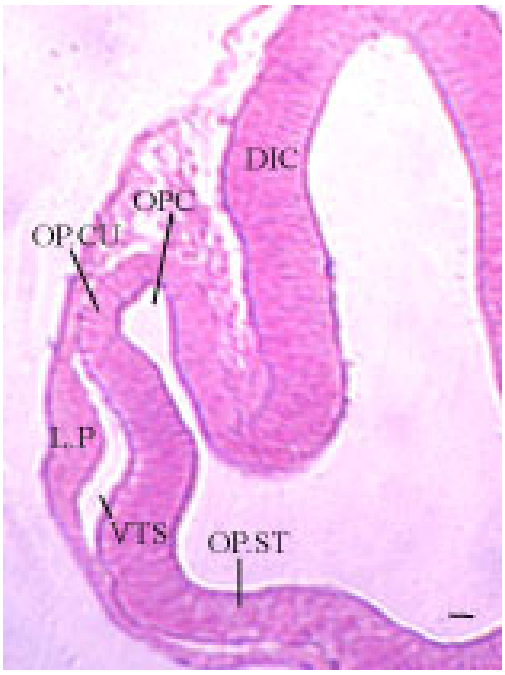

Figure-2
At 1.2 cm total body length, showing developing lens placode (L.P), optic cup (OP.CU), viterous space (VTS). Scale bars,25µm

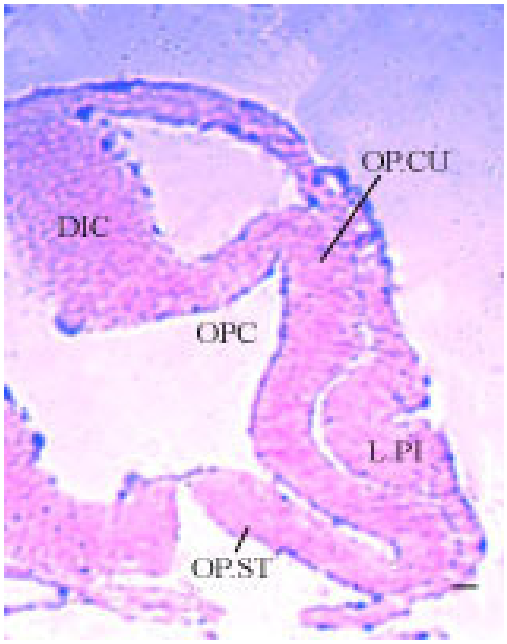

Figure-3
At 1.2 cm total body length, showing forming lens pit (L.PI). Scale bars,25µm.

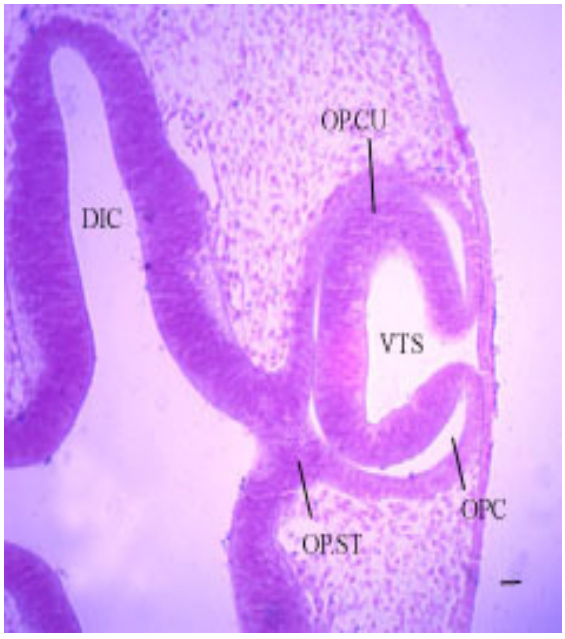

Figure-4
At 1.5 cm total body length, showing a double layer optic cup, with well developing viterous space with minimize optocoele. Scale bars,250µm.

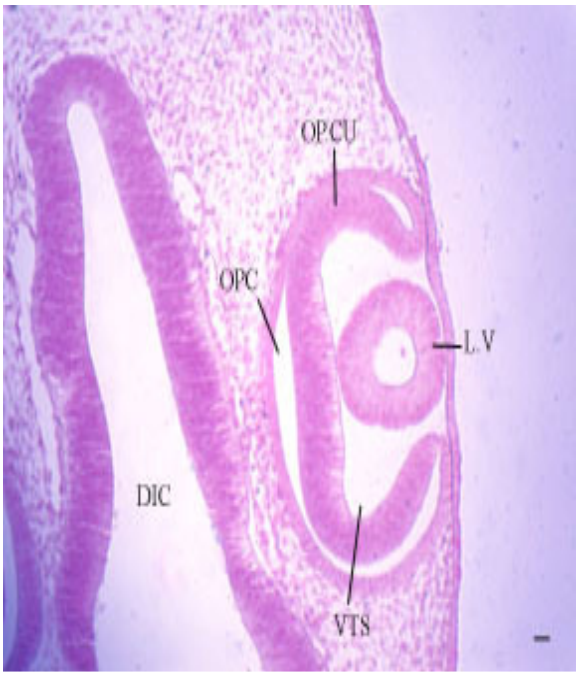

Figure-5
At 1.5 cm total body length, showing rounded lens vesicle, well organized viterous space. Scale bars, 250µm

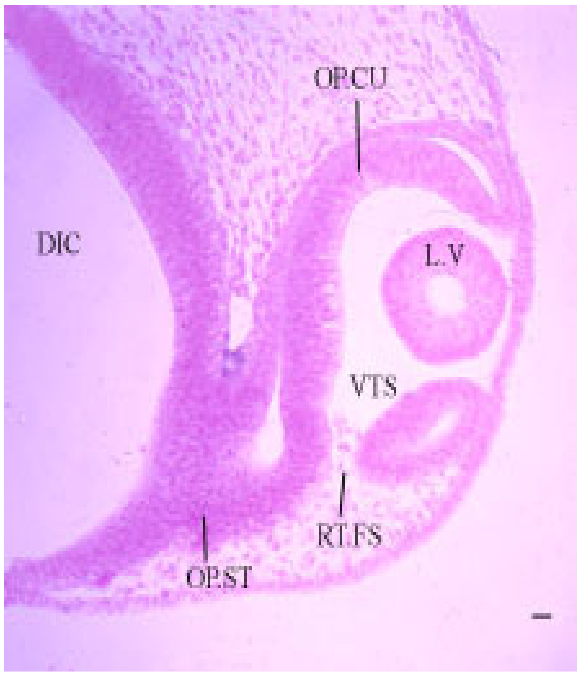

Figure-6
At 1.9 cm total body length, showing evidence of retinal fissure (RT.FS). Scale bars, 25µm

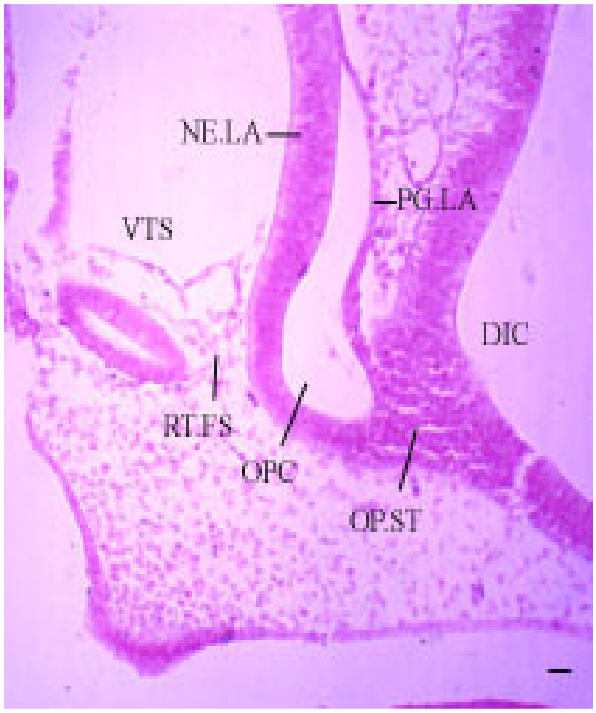

Figure-7
At 1.9 cm total body length, showing future outer thin pigmented layer of the retina (PG.LA), and future inner thick neural layer of the retina (NE.LA). Scale bars, 250µm.

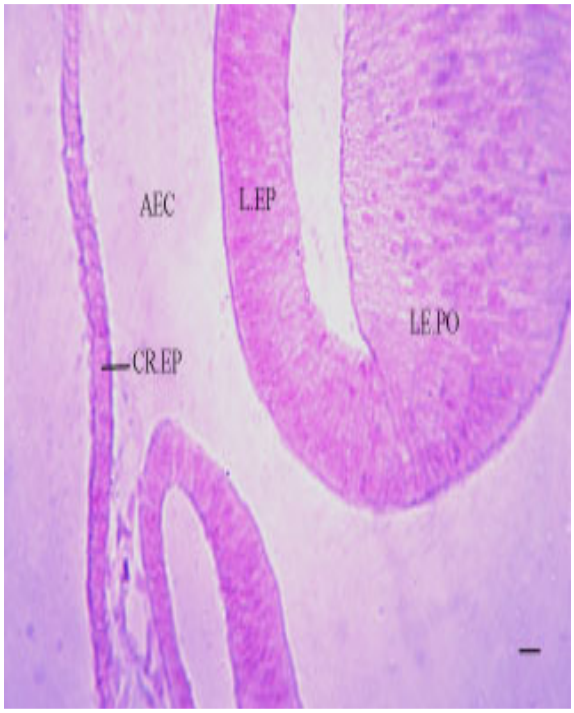

Figure-8
At 1.9 cm total body length, showing anterior eye chamber (AEC), future corneal epithelium (CR.EP), the lens vesicle formed from lens epithelium (L.EP), and lenticular portion (LE.PO). Scale bars, 104**µm.**

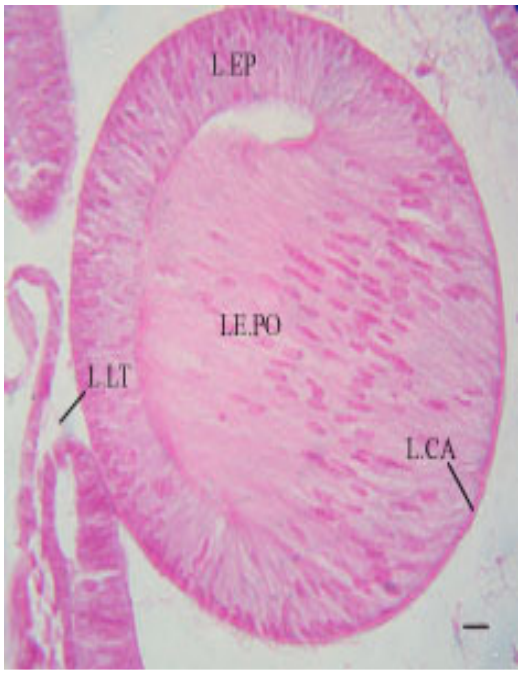

Figure-9
At 4 cm total body length, showing lens capsule (L.CA), lens ligament (L.LT). Scale ars,14.7µm.

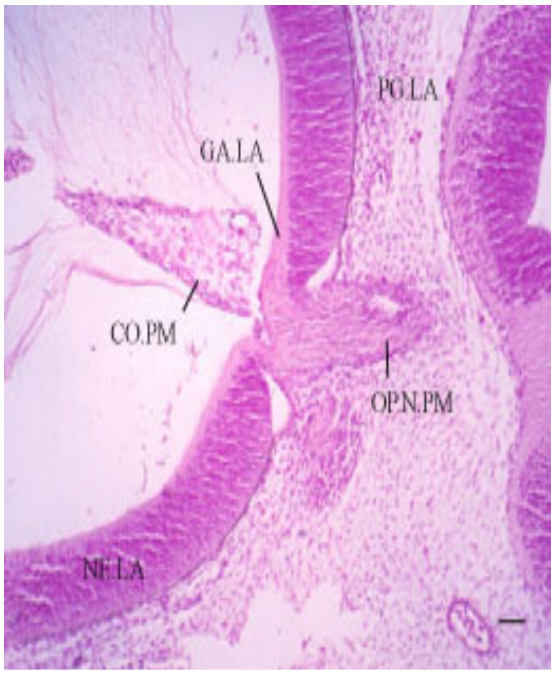

Figure-10
At 5 cm total body length, showing appearance of conus papillaris primordium (CO.PM), ganglionic layer (GA.LA) of the inner neural layer appearing, thick inner neural layer, developing optic nerve primordium (OP.N.PM), pigmented granules in outer pigmented layer. Scale bars, 41.6µm

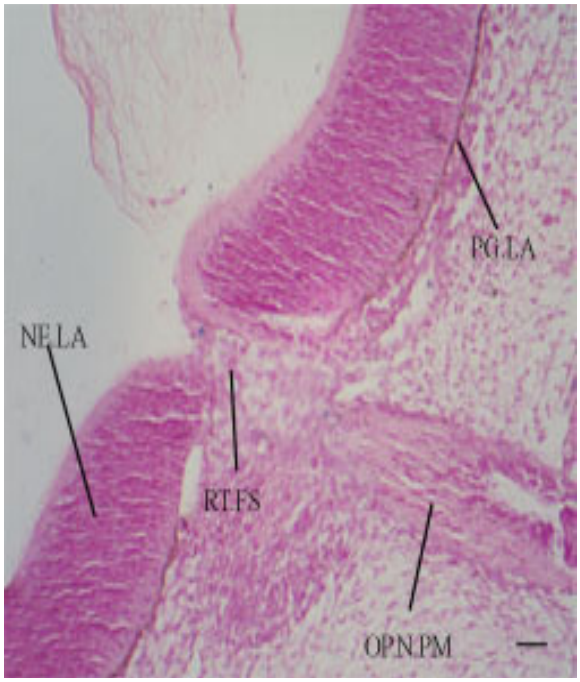

Figure-11
At 5 cm total body length, showing appearance of closure of retinal fissure invaded with mesenchymal element. Scale bars, 6.25µm

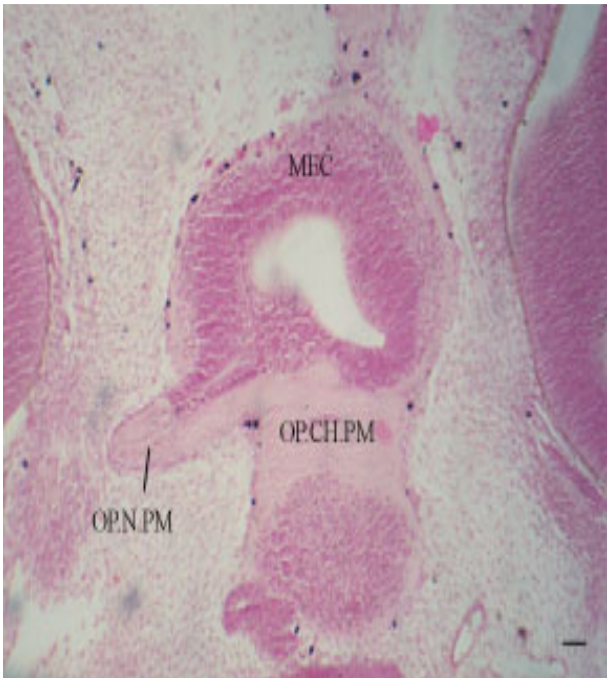

Figure-12
At 5 cm total body length, showing appearance of optic chiasma primordium (OP.CH.PM). Scale bars, 41.6µm

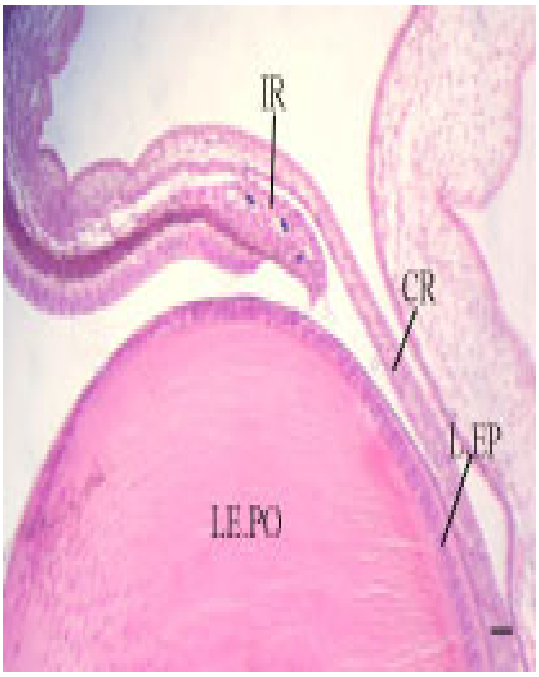

Figure-13
At 5 cm total body length, showing appearance of primordium of iris and ciliary process (IR.PM), corneal primordium layers differentiation. Scale bars, 25µm

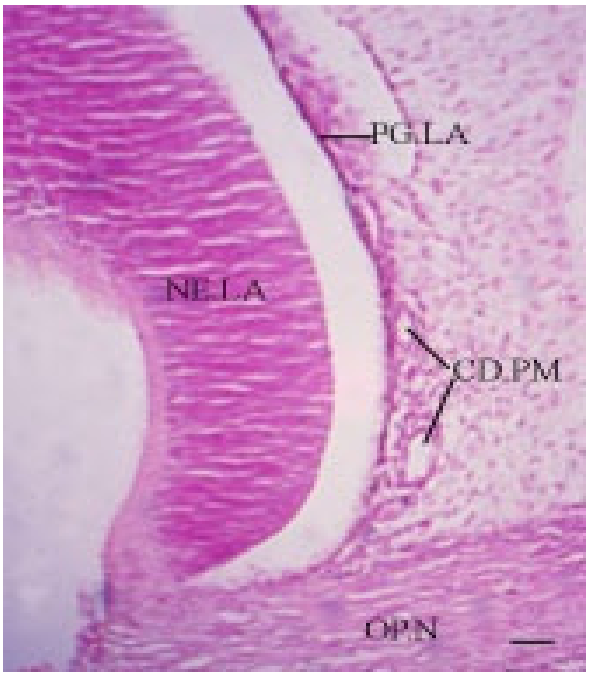

Figure-14
At 5 cm total body length, showing appearance of primordium of choroid (CD.PM), optic nerve appearance (OP.N). Scale bars, 250 µm

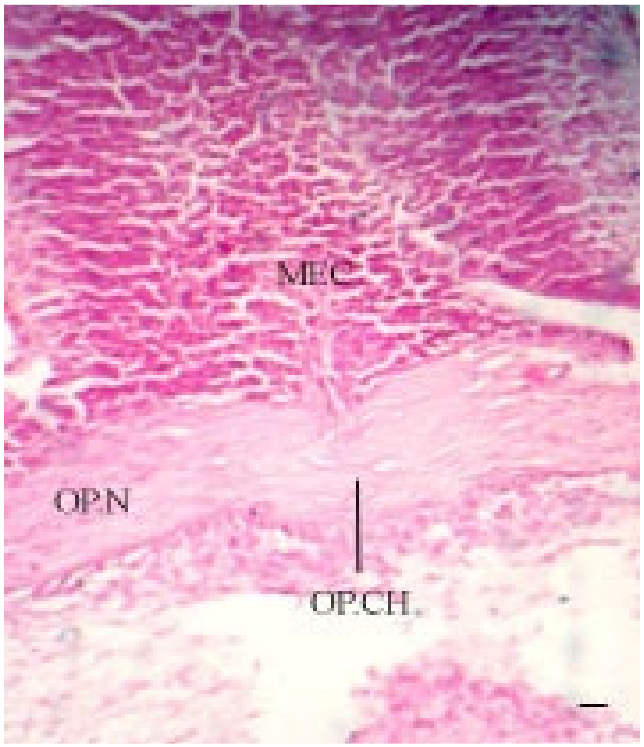

Figure-15
At 5.5 cm total body length, showing appearance of optic chiasma (OP.CH). Scale bars, 250µm

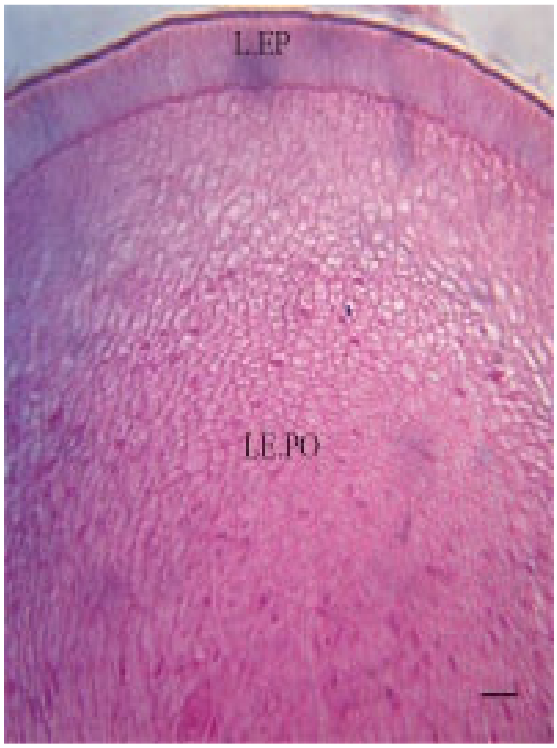

Figure-16
At 5.5 cm total body length, showing thin lens epithelium of the lens vesicle, crystalline lenticular portion of the lens. Scale bars,250µm.

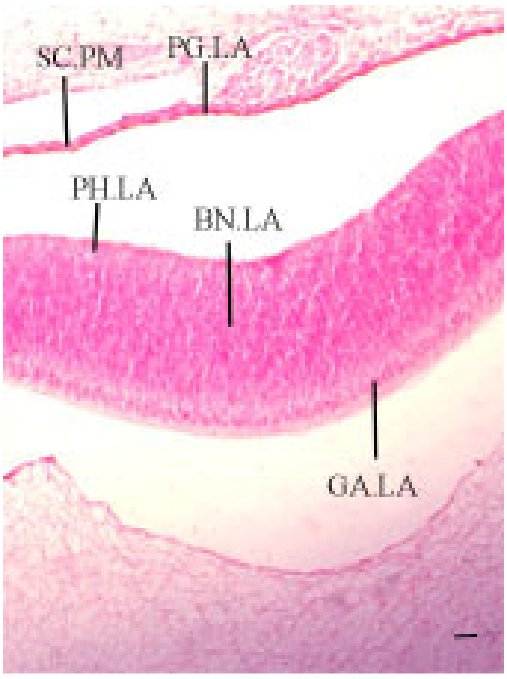

Figure-17
At 6.5 cm total body length, showing differentiates of neural retina into an outer layer of photoreceptor (PH.LA), a middle thick layer of bipolar neurons (BN.LA), an inner ganglionic layer, appearance of sclera primordium (SC.PM). Scale bars,50µm.

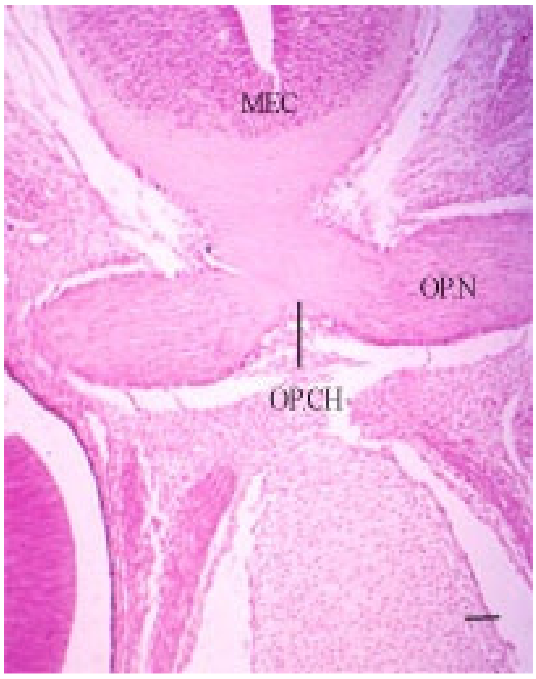

Figure-18
At 6.5 cm total body length, showing a well developed optic chiasma. Scale bars,50µm.

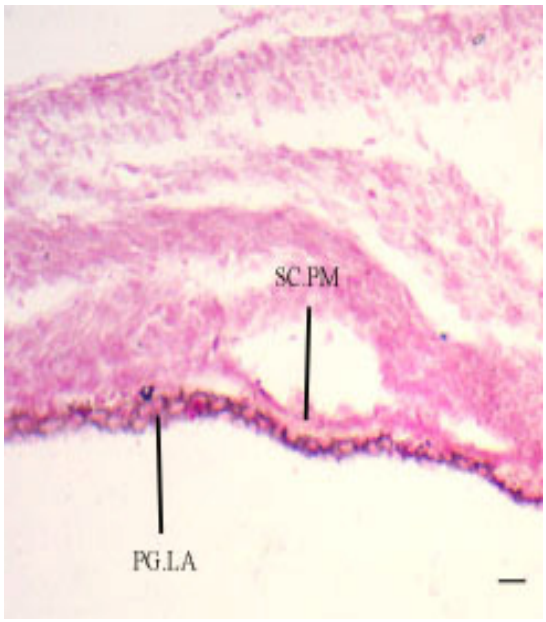

Figure-19
At 6.5 cm total body length, showing high degree of pigmentation of the outer pigmented layer, fibrous sclerotic primordium. Scale bars,12.5µm.

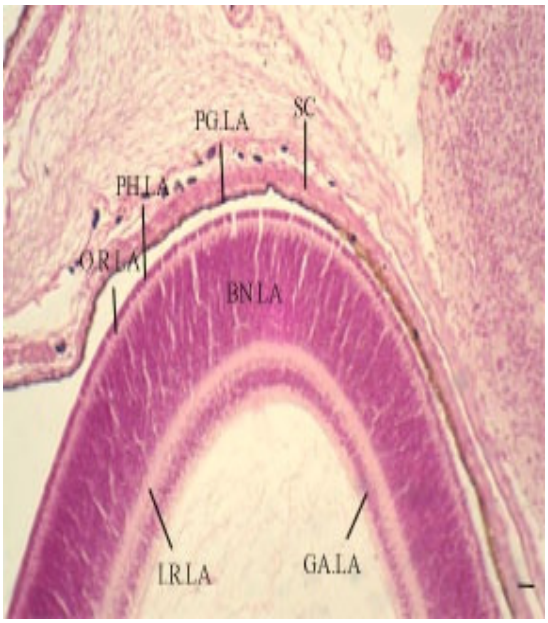

Figure-20
At 7 cm total body length, showing the neural retina consists of six layers: from outside to inside: a photoreceptor layer, the outer reticular layer (O.R.LA), a bipolar neural layer, an inner reticular layer (I.R.LA), a ganglionic layer , a nerve fiber layer, more developing sclera (SC). Scale bars,25µm.

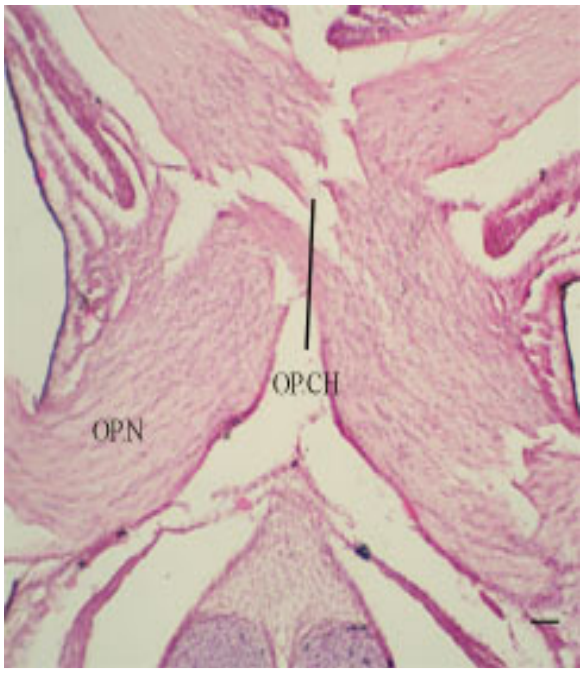

Figure-21
At 7 cm total body length, showing growing optic chiasma. Scale bars,6.25µm.

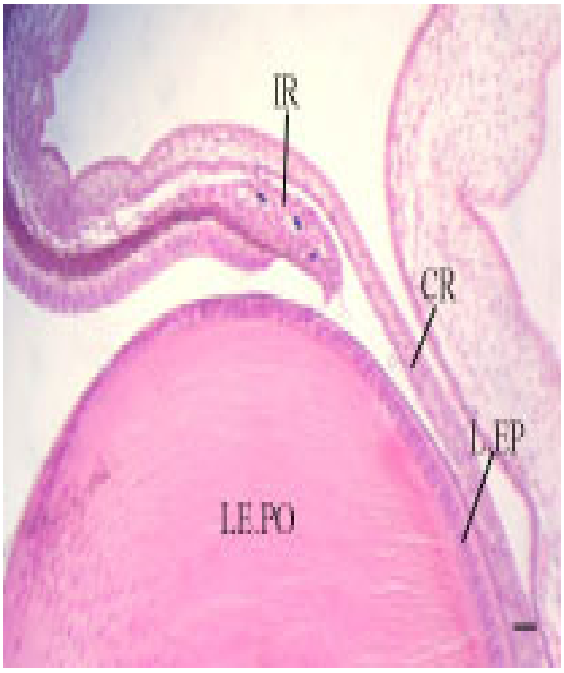

Figure-22
At 7 cm total body length, showing developing iris (IR), cornea (CR). Scale bars,25µm.

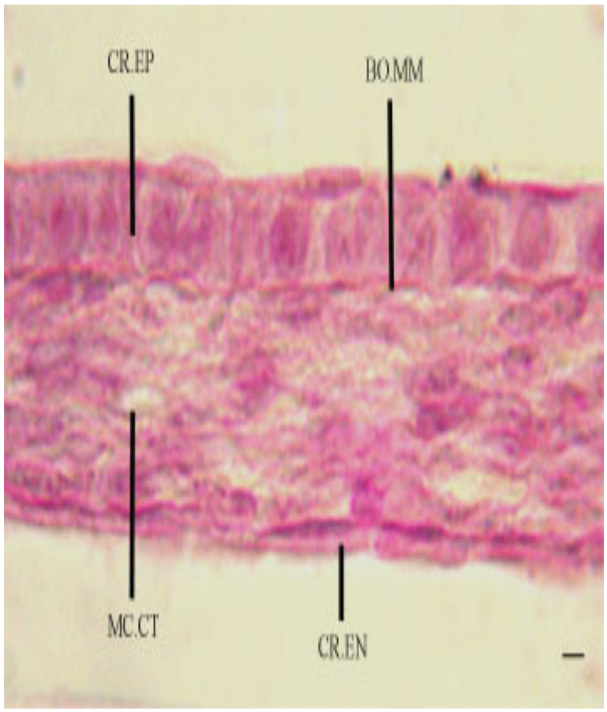

Figure-23
At 7 cm total body length, showing the developing cornea (Fig.23) consists of four layers: the corneal epithelium, Bowman′s membrane primordium (BO.MM), a layer of mesenchymal connective tissue (MC.CT), the inner most layer is the corneal endothelium (CR.EN). Scale bars6.25µm.

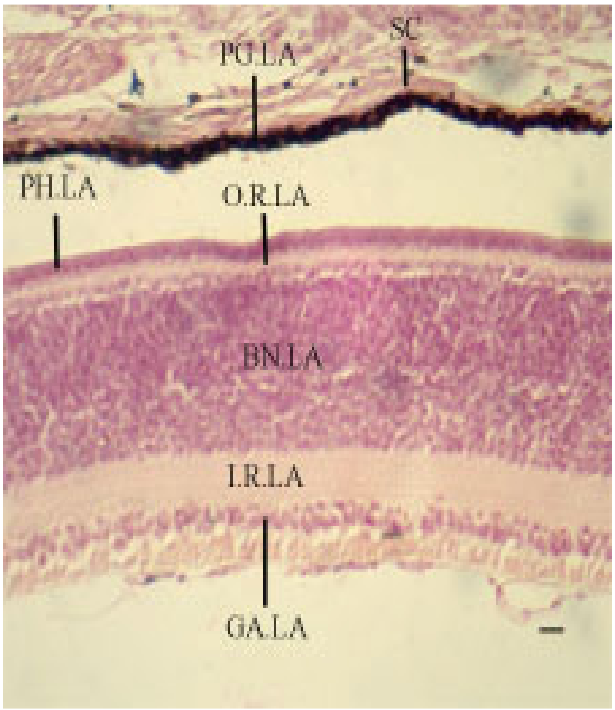

Figure-24
At 8 cm total body length, showing the developing retina. Scale bars, 25µm.

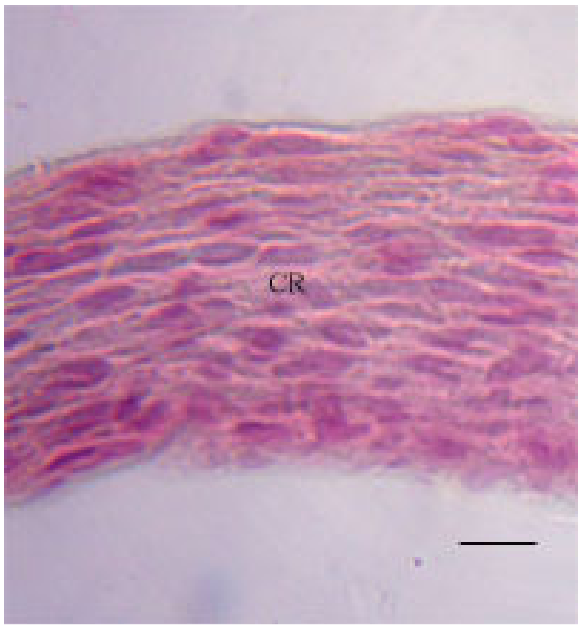

Figure-25
At 8 cm total body length, showing poorly differentiated cornea. Scale bars, 6.25µm

Stage 10 (Total body length: 8cm): The developing retina figure-24 becomes more developed than the previous stage. The outer pigmented layer (PG.LA) of the retina consists of two layer of cuboidal cells that shows a high degree of pigmentation. Where, the outer reticular layer (O.R.LA) and an inner reticular layer (I.R.LA) of the neural retina increase in its thickness, the bipolar neural layer (BN.LA) of the neural retina decrease in its thickness.In *Malpolon*, the cornea (fig-25 CR, 25) was poorly differentiated. Where, all the cells of it are squamous in its form.

Discussion: The investigation of the morphological properites of the eye of venomous rear-fanded Colubrids snake; *Malpolon monspessulanus* during different developmental stages may present information on its visual potential. Also, anatomical investigations give setting awareness for study of taxonomy, inaddition first information to talk about and understand the evolution in biology. In addition, this study describes the morphological changes as a baseline for clinically ophthalmic structures in this species.

Eye of *Malpolon* starts its development as a lateral protrusion of the fore brain called the primary vesicle, which invaginates to form a double wall secondary optic cup. Organogenesis of eye is a multi successive steps that begins with the development of optic vesicle followed by inward growth of the inner wall of the vesicle and the opposite lens placode resulting in organization of the optic cup[15].

Difference in duration of appearance of eye structures are varied in different species, in *Malpolon* optic vescicle appears in 1 cm total body length. While, the primary eye vesicles in anamniotes start to appear at the 2.0 mm stage of the frog embryo[16] and in amphibian; at the second day stage of development in frog, optic cup appears[16,] at 2.0mm stage in the bony fish *Cyprinus carpio* the primary optic vesicle appears[17]. In the 8.9mm stage in the Oman shark the primary vescicle appears[18].

In some mammals, as in the mouse, the primary optic vesicle evaginates at 9-10 days[19] or at 8.5 days[20]. The optic vesicle transformation into an optic cup varies among species. In the mouse, the optic cup (retinal primordium) appears at the 9.5 day stage of development[20]. On the 22 day of human's embryo, the eye vesicle appears[21, 22].

In *Natrix natrix*, the optic nerve consists mainly of fibres of retinal origin, and has a small number of efferent fibres. Moreover, most of the retinal fibres decussate forming an optic chiasma, but a small proportion of fibres remain uncrossed[23], our data supports the result which found in *Natrix*. In *Malpolon*, the optic stalk appears at 1 cm total body length. In *Xenopus laevis*, the optic stalk firstly appears at stage-3[24]. At 22 days in human, the optic stalk develops[22].

In this current study supports this fact; as in vertebrates at the beginning of growth, the retina formed from homogeneous single-layered neuroepithelium cells. As maturation proceeded, retina differentiated into well defined laminated tissue[25].

In *Malpolon*, the vascular conus appear as a group of fibres that pass from papilla to be lost in the vitreous humour. While, in *Viper berus*, the vascular conus is best developed and heavily pigmented. Small, non-vascular, and pigmented conus present in *Eristocophis*. Well developed and unpigmented conus in *Lampropeltis*. A number of snakes, such as *Epicrates* and *Coronella*, have conus in embryonic stages that is atrophied or lost in mature stage[26]. The latter information about the conus is achieved in the current in vesrtigation

The ectoderm forms the lens placode primordium. The latter comes in contact with the primary optic vesicle at the 1.0 cm total body length stage. Where in this investigation, the ectodermal cells at this region become elongated, thicker forming placode of the lens[27]. What is worth to mention, the lens placode differes than all cranial placode that is not form neurons and share with all cranial placode that it arise from ecodermal layer near to the neural plate[28].

It is worthy to mention that in amphibians the lens vesicle is formed in a different manner**.** At the time of hatching, the lens placode constricts off as a solid rounded mass of cells, which attains a cavity forming the lens vesicle that is soon closed by the thickening of the cells of the future retinal side[16].

The lens capsule of *Malpolon* as mentioned in results appears in late developmental stage, before the lens vesicle becomes away from the ectoderm, the lens capsule can be first seen as a darkly stained line on the medial side of lens vesicle[19]. The lenticular cells share in the formation of the lens capsule[29].

In the current study of *Malpolon*, the anterior eye chamber is well defined. While in both of *Amphisbaena alba* and *Typhlops brongersmianus* the anterior eye chamber is absent as an evidences of fossorial adaption[6]

The substantia propria of the cornea and corneal endothelium are formed by the mesoderm forms[22] .This agrees with the current study. The cornea of *Malpolon* in more developing stage is well organized while in *Amphisbaena* and *Typhlops*, the cornea formed from a single layer of epithelial cells, so it characterized as thin and rudimentary structure[6].

Our investigation emphasized the sclera of the studied species is not cartilaginous. Also, the sclera of *Typhlops brongersmianus* has not observed cartilage element in the scleral. In contrast with our data, the sclera of *Amphisbaena alba* is cartilaginous in its structure[6].

The development of the human iris terminates much earlier than that of the human retina[30]. These finding agrees with our study.

Conclusion

A new phylogenetic data can be obtained from great embryological variation of an optic development of squamata.

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