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A histological study of the eye in *Hyla orientalis* (Bedriaga, 1890) (Anura, Hylidae)

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Abstract. Amphibian eyes have an important role in vision and in several physiological processes, such as feeding and locomotion. In the present study, the eye of *Hyla orientalis* was investigated in terms of histological structures. Light microscopic observations revealed that the eye of *H. orientalis* was formed by three layers from outside to inside: (a) outer layer- the sclera and the cornea; (b) middle layer- the choroid, ciliary body and the iris; and (c) inner layer- the retina. The cornea was composed of well-defined layers, including a squamous corneal epithelium, the stroma, and corneal endothelium. The lens of *H. orientalis* was spherical and enclosed by a lens capsule, and its anterior surface was covered by a monolayer of epithelial cells. The posterior sclera consisted of a cartilaginous cup, embedded within a collagenous matrix unlike terrestrial animals. The retina was avascular, and layers of the retina conformed in principle to the standard vertebrate arrangement.

Key words: amphibian, retina, cartilaginous cup, cornea, sclera, histology.

Introduction

In vertebrate evolution, the transition from aquatic to terrestrial environment has been a critical episode. This fact provides scientific interest to researchers due to the fact that amphibian adaptations enables the use of both aquatic and terrestrial habitats (Izecksohn & Carvalhore-e-Silva 2001, Santana et al. 2005).

The eyes are an important organ in many animal species. Because the morphology and physiology of this organ reflect the animal's life style and environment (Duellman & Trueb 1994). The eyes of amphibians vary greatly in size. Because the eyes are involved in behaviour and selection of refuge (Kerr et al. 2003, Auburn et al. 2009), food choice (Wohlfeil 2008), orientation and spatial position (Freake 2001) and the detection of approaching threats (Murray & Bull 2004). Additionally, the eyes play a role in other physiological processes such as breathing movements, gulping down the air into the lungs, and sound production. Anurans depend on vision for feeding and locomotion. They have the largest and most developed eyes among amphibians (De Jongh & Gans 1969, Morandini 1976, Duellman & Trueb 1994, Santana et al. 2005).

Amphibians are prominent vertebrate group in terms of a study model in physiology, and this group is a standard for the study of many biological processes (Feder 1992). However there are very limited study about amphibian ocular morphology and histology. The aim of this study was to examine the ocular morphology of *Hyla orientalis* and compare several aquatic and terrestrial vertebrates.

Material and Methods

Five specimens (three adult males and two adult females) of *H. orientalis* were captured from Menderes-İzmir/Turkey (N 38°15'14'' and E 27°8'2''). They were euthanized and for histological investigation, each eye was dissected free from the orbit and fixed with Bouin's fluid for 48 h at 4ºC and washed with ethanol 70% for 24 h. Dehydration was carried out in upwards gradient ethanol baths (95%, 100%). Tissues were embedded in paraffin, five micrometers serial sections were stained with Gill's hematoxylin-eosin (modified Gill et al. 1974) following standard protocols, and then sections were photographed using a Leica DM3000 microscope (Leica Microsystems) that was equipped with a Leica digital camera (DFC290).

Results

The *H. orientalis* eye was formed by three layers from outside to inside: (a) outer layer- the sclera and the cornea; (b) middle layer- the choroid, ciliary body and the iris; and (c) inner layer- the retina. The cornea was comprised of a squamous corneal epithelium, the stroma, and corneal endothelium. The epithelium was a layer of cells that covered the surface of cornea. The stroma was the thickest layer of cornea and composed of tiny collagen fibrils run parallel to each other. The lens was made up of three layers, an outer acellular sheath, monostratified cuboidal epithelial cell layer and an innermost fibrous layer (Fig 1). The sclera consisted of dense bundles of collagen fibers. The anterior sclera formed cornea. The ciliary epithelium was composed of the inner non-pigmented layer and outer pigmented layer (Fig 2).

Light microscopy observations revealed that the posterior sclera consisted of a cartilaginous cup, embedded within a collagenous matrix. The cup-shaped cartilage piece displayed the basic histological structure of hyaline cartilage. At the periphery, the sclera exhibited a typical perichondrium, which is formed by a fibrous connective tissue. At the retinal side, the cartilage was not cover by a perichondrium, and the cartilage matrix displayed an intimate relationship with choroid. Choroid was located between the cartilage and retina (Fig 3).

The retina was avascular and layers of the retina conformed in principle to the standard vertebrate arrangement. The retina was divided into layers; pigment epithelium layer, photoreceptor cell layer, outer limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer and nerve fiber layer. The photoreceptor layer contained densely packed, elongated processes embraced by the retinal pigment epithelium The inner nuclear layer was much thicker than its outer counterpart, consisting of loosely organized cells in the central retina. Further vitreal, the inner plexiform layer formed a meshwork of neuronal processes connecting the inner nuclear layer with the ganglion cell layer (Fig 4).

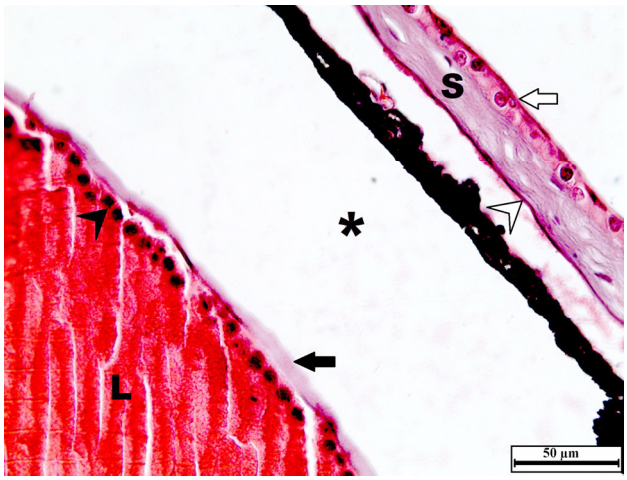

Figure 1. Transverse section of cornea and lens of *Hyla orientalis* stained with hematoxylin-eosin method (HE). (Bar= 50 µm). The lens of *H. orientalis* was enclosed by a lens capsule, and its anterior surface was covered by a monolayer of epithelial cells. Squamous corneal epithelium (white arrow), corneal stroma (Bowman's membrane) (S), corneal endothelium (white arrow head), aqueous chamber (*), acellular lens capsule (black arrow), cuboidal lens cells (black arrow head), lens (L)

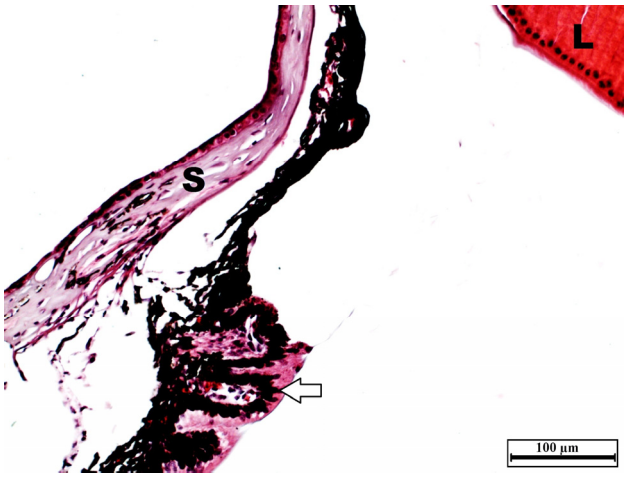

Figure 2. Iris, sclera and lens stained with HE, transverse section (Bar= 100 µm). Capillaries surrounded by pigmented epithelia bordered the anterior margin of the iris. The sclera consisted of dense bundles of collagen fibers. Sclera (S), lens (L), ciliary body (white arrow).

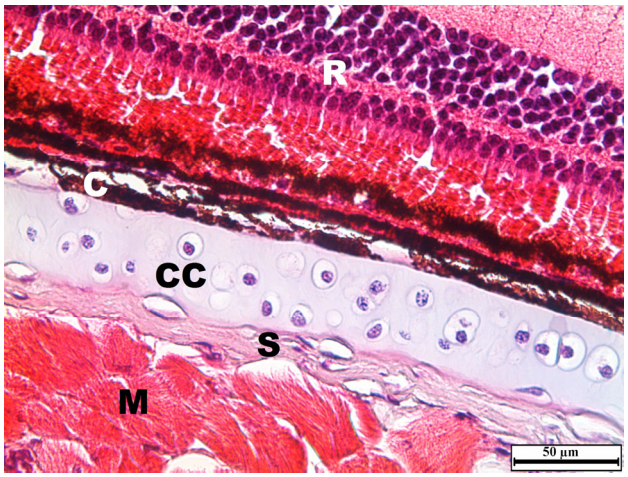

Figure 3. Cartilaginous cup was located between choroid and sclera, transverse section, HE (Bar= 50 µm). Muscle (M), sclera (S), cartilaginous cup (CC), choroid (C), retina (R).

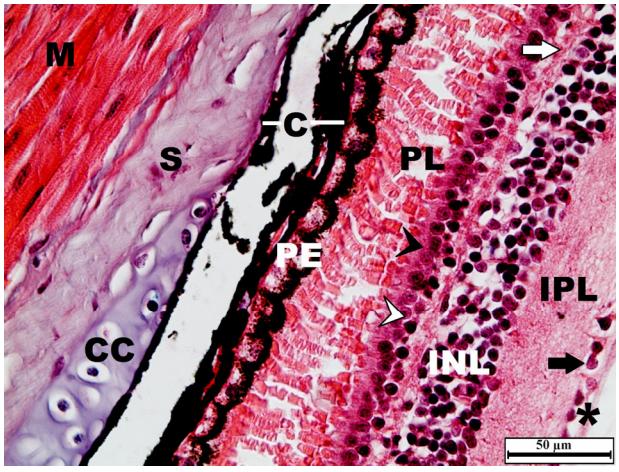

Figure 4. Light micrograph of the retina in *H. orientalis*, transverse section, HE (Bar= 50 µm). The retina layers: pigment epithelium layer (PE), photoreceptor layer (PL), outer limiting membrane (black arrow head), outer nuclear layer (white arrow head), outer plexiform layer (white arrow), inner nuclear layer (INL), inner plexiform layer, (IPL), ganglion cell layer (black arrow), nerve fiber layer (*). The posterior sclera consisted of a cartilaginous cup, embedded within a collagenous matrix. Muscle (M), sclera (S), cartilaginous cup (CC), choroid (C).

Discussion

The eye of vertebrates is essential for feeding, mating and survival. Additionally, vertebrates inhabit a wide spectrum of habitats including aerial, terrestrial and aquatic ones, which may have different demand on the structure of eye (Collin & Collin 1998). The structure of eye has been extensively reported for humans (Beuerman & Pedrosa 1996) and various vertebrates, including monkey (Svedbergh & Bill 1972, Sugita & Nakano 1980), rat (Yee et al. 1987) and fish (Soules & Link 2005, Mansoori et al. 2012). However there is little previously published information describing the ocular

morphology of amphibians.

The *H. orientalis* eye was formed by three layers from outside to inside, as observed in vertebrates: outer layer (the sclera and the cornea), middle layer (the choroid, ciliary body and the iris) and inner layer (the retina). The vertebrate eye develops three distinct embryological tissues: neuroectoderm, ectoderm and mesenchyme. The neutral retina, pigmented epithelium, optic stalk and ciliary margin are derived from neuroectoderm, ectoderm which forms the lens and cornea, the neural crest cell (NCC) derived periocular mesenchym gives rise to the cornea and sclera (Trainor & Tam 1995, Cvekl & Tamm 2004, Fadool & Dowling 2008). The anterior part of the vertebrate eye is composed of the cornea, lens, iris, ciliary body. The anterior part of eye is responsible for focusing of the incoming light onto the neural retina and regulating intraocular pressure. In all vertebrates, the balance between aqueous humor production and outflow provide for maintenance of intraocular pressure (Wall 1942, Leonard & Meek 1997, Gabelt & Kaufman 1997, Soules & Link 2005).

The lens of *H. orientalis* was enclosed by a lens capsule, and its anterior surface was covered by a monolayer of epithelial cells. It had typical vertebrate composition with two cell types, lens epithelial cells and lens fiber cells. The lens of *H. orientalis* was relatively large and spherical like zebrafish lens (Soules & Link 2005) and Leatherback sea turtle (*Dermochelys coriacea*) lens (Brudenall et al. 2008). However Lluch et al. (2008) reported the lens of some wild rodents (*Apodemus sylvaticus*, *Mus domesticus, M. spretus*, *Clethrionomys glareolus, Arvicola terrestris, Microtus arvalis*) was a biconvex body with the anteroposterior diameter smaller than the equatorial diameter.

The posterior sclera of *H. orientalis* consisted of a cartilaginous cup, embedded within a collagenous matrix. A typical cartilage piece was visualized between the sclera and the choroid in *Bufo ictericus* and *Rana catesbeiana* (Santana et al. 2005). Brudenall et al. (2008) observed that the sclera of Leatherback sea turtle (*D. coriacea*) consisted of a cartilagious cup embedded within a collagenous matrix. Mansoori et al. (2012) reported that two cartilaginous segments with connective tissue correlation were seen in the sclera of rabbit fish (*Siganus javus*) eye. Lluch et al. (2008) detected the eye morphology in some wild rodents (*A. sylvaticus*, *M. domesticus, M. spretus, C. glareolus, A. terrestris, M. arvalis*), and cartilaginous segment was not observed. There is probably correlation between animal habitats, activity of eye and presence/absence of cartilaginous segment (Mansoori et al. 2012).

The retina of *H. orientalis* was avascular, and arrangement of retinal layers was similar to other vertebrates. The neuroretinal layers included a clearly defined photoreceptor layer, outer and inner nuclear layer, outer and inner plexiform layers and ganglion cell and nerve fibre layer like in *D. coriacea* (Brudenall et al. 2008), *Tiliqua rugosa* (New et al. 2012) and some wild rodents (Lluch et al. 2008).

Consequently, this study revealed ocular morphology and histology of *H. orientalis* with comparison of several aquatic and terrestrial vertebrates. The morphology of visual system of *H. orientalis* may be considered important feature for developmental, evolutionary and ecological studies.

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