Anatomical, scanning electron microscopic, and histological studies of the ocular lids and muscles of the Egyptian agama Trapelus mutabilis

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September 23, 2024

Abstract

The present study aimed to describe the anatomical, scanning electron microscopic, and histological features of the ocular eyelids and muscles of the Egyptian agama, *Trapelus mutabilis*. The eye of the Egyptian agama is characterized by mobile upper and lower eyelids and a small, immobile, nictitating membrane. The upper eyelid appears shorter than the lower one, and the head skin above the upper eyelid extends laterally to form a superior extension. The scales of the eyelids are arranged in the same order and are equipped with micro-ornamentation and sense organs at their tips. In contrast, the histological structure of the upper eyelid is similar to that of the lower eyelid. The external surface of both eyelids is composed of keratinized stratified squamous epithelium of 2-4 cell layers, while cuboidal stratified epithelium lines their internal surface. Both eyelids contain iridophores and melanophores. The immobile nictitating membrane (third eyelid) appears as a small protrusion with a concave surface that connects with the lacrimal gland posteriorly at the anterior canthus of the eye. Its external surface is covered with stratified squamous epithelium, while its internal surface is lined by one or two layers with rounded nuclei attached to the conjunctival epithelium. Video recordings of eye movement in the laboratory revealed that the movement of the eyelid is synchronized with the eyeball movements, allowing all eye movements to be observed. The placement of the eye inside the skull, its protection by two eyelids equipped with hard scales and sensory organs, and the presence of a superior extension of broad scales acting as an umbrella for the eyes, in addition to the presence of two types of pigment cells, enhance eye protection against ultraviolet rays. All these characteristics are well-suited for the remote desert environment in which the agama lives.

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Acknowledgements

The authors are thankful to Faculty of Science, Assiut University, Egypt, for providing the opportunity to conduct this work, and electron microscopy units for assistance with SEM investigations.

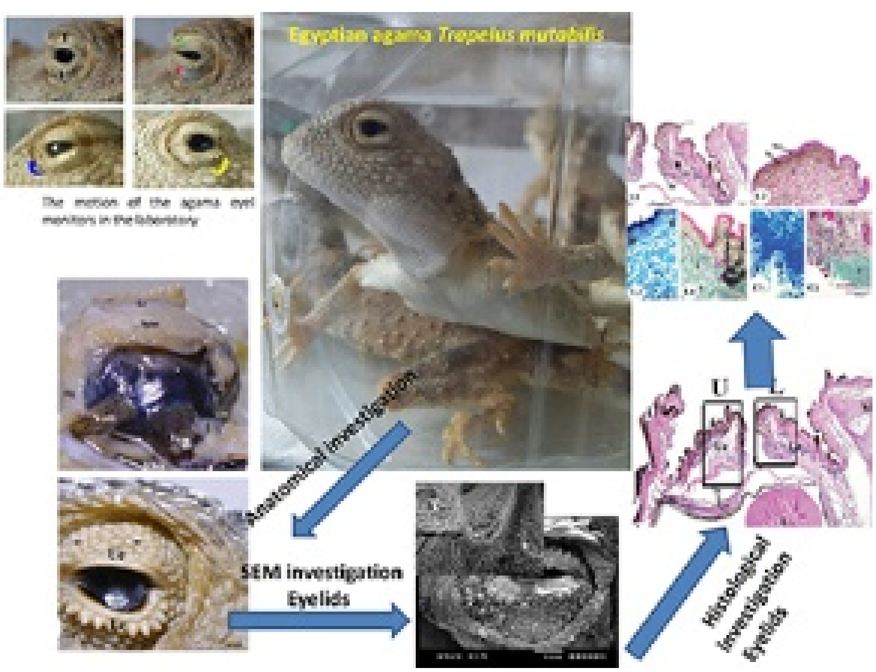
Abstract

The present study aimed to describe the anatomical, scanning electron microscopic, and histological features of the ocular eyelids and muscles of the Egyptian agama, *Trapelus mutabilis* . The eye of the Egyptian agama is characterized by mobile upper and lower eyelids and a small, immobile, nictitating membrane. The upper eyelid appears shorter than the lower one, and the head skin above the upper eyelid extends laterally to form a superior extension. The scales of the eyelids are arranged in the same order and are equipped with micro-ornamentation and sense organs at their tips. In contrast, the histological structure of the upper eyelid is similar to that of the lower eyelid. The external surface of both eyelids is composed of keratinized stratified squamous epithelium of 2-4 cell layers, while cuboidal stratified epithelium lines their internal surface. Both eyelids contain iridophores and melanophores. The immobile nictitating membrane (third eyelid) appears as a small protrusion with a concave surface that connects with the lacrimal gland posteriorly at the anterior canthus of the eye. Its external surface is covered with stratified squamous epithelium, while its internal surface is lined by one or two layers with rounded nuclei attached to the conjunctival epithelium. Video recordings of eye movement in the laboratory revealed that the movement of the eyelid is synchronized with the eyeball movements, allowing all eye movements to be observed. The placement of the eye inside the skull, its protection by two eyelids equipped with hard scales and sensory organs, and the presence of a superior extension of broad scales acting as an umbrella for the eyes, in addition to the presence of two types of pigment cells, enhance eye protection against ultraviolet rays. All these characteristics are well-suited for the remote desert environment in which the agama lives.

Keywords: agama, eyes, pigment cells, ultraviolet, video –recordings

Research Highlights and Graphical Abstract

The hard life of Egyptian agama has attracted the attention of the present authors, and how these animals maintain their visual performance under such these difficult living conditions. Studying the structure of the eyelids helps to understand some information about the visual performance of these animals. The present study has shown that all the eyelids characteristics that mentioned in this study are well-suited for the remote desert environment in which the agama lives.



Introduction

The ocular adnexa are the protective and supporting structures of the eye, including the eyelids and their parts, the third eyelid (nictitating membrane), orbital glands, and oculomotor muscles (Guerra-Fuentes et al., 2014). Peripheral to the housing of the orbit, the eye receives immediate protection and support from the eyelids (Franz-Odendaal and Vikaryous, 2006). All reptiles have external eyelids. Both upper and lower eyelids are present in most lizards, all turtles, tuataras, and crocodilians. Eyelids are modified in several species, resulting in partial fusion, as in chameleons, leaving a circular opening equivalent to the diameter of the cornea, or complete fusion with transparency, as in many geckos and snakes (Schwab, 2012). In snakes, the eyelids fuse during development to form the spectacle. Some gecko and skink species possess a secondarily derived spectacle. Additionally, some skinks, lacertid lizards, and iguanine lizards have a transparent lower eyelid formed by clear scales (Lawton, 2006; Schwab, 2012)\RL.

The nictitating membrane is a prominent semilunar fold of the conjunctiva situated at the anterior canthus of the eye, between the eyelids and the cornea (Schlegel et al., 2003). It is highly developed in crocodilians and turtles but is absent in snakes and many lizards (Lawton, 2006)\RL. Hiller (1995, 1998) studied the ultrastructure of the conjunctiva and nictitating membrane of the eyelids in agamid lizards and other reptilian species through scanning electron microscopy. Starostová et al. (2009, 2013) examined cell size and its effects on the scaling of metabolic rate in eyelid geckos, finding that ontogeny of metabolic rate and red blood cell size in these species follow different paths. Guerra-Fuentes et al. (2014) investigated the spectacle in gymnophthalmid lizards through the looking glass.

The study of extraocular muscles has received little attention from reptilian anatomists, as they consider these muscles' function and innervation patterns to be standard among vertebrates. Vertebrate extraocular muscles consist of six muscles that typically control the movement of the eyeball (Nomina Anatomica Veterinaria, 2012). These muscles are named according to their insertion points and form three pairs (antagonistic and synergistic) during eyeball movement (Cunha et al., 2016)\RL.

Ocular movement varies among species, ranging from more than 270 degrees of rotation in chameleons to a few degrees in snakes (El Hassni et al., 2000). Chameleons' highly independent eye movements, which differ from those of other reptiles, have garnered significant interest from many researchers. Pettigrew et al. (1999) studied the convergence of specialized behavior, eye movements, and visual optics in the sand lance (Teleostei) and the chameleon (Reptilia). El Hassni et al. (2000) investigated the localization of motor neurons innervating the extraocular muscles in the chameleon (*Chamaeleo chameleon*). Ott (2001) found that chameleons have independent eye movements but synchronize both eyes during saccadic prey tracking\RL.

Reptilian anatomists have focused more on studying the eye structure of certain reptilian species, such as chameleons and snakes, in detail, but some species, like the Egyptian agama, remain neglected. The Egyptian agama, *Trapelus mutabilis* , is a small diurnal lizard found in North Sinai, Egypt (Baha el Din, 2006; Wagner et al., 2011). The hard life of Egyptian agama has attracted the attention of the present authors, and how these animals maintain their visual performance under such these difficult living conditions. Studying the structure of the eyelids helps to understand some information about the visual performance of these animals. This study presents the anatomical, scanning electron microscopic and histological investigations of the ocular eyelids and muscles of the Egyptian agama, *Trapelus mutabilis* . Additionally, the motion of the agama eye was monitored in the laboratory to observe the synchronization between eyelid and eyeball movement\RL.

Materials and Methods

Ten adult Egyptian agama lizards, *Trapelus mutabilis* , were collected from North Sinai, Egypt, during April and May. Live samples were brought to the Comparative Anatomy of Vertebrates Laboratory and dissected according to the guidelines of the research ethics committee at Aswan University (Approval number: ASWU/05/SC/ZO/23-03/01)\RL.

For anatomical study , the sample heads were fixed in 10% formalin for two weeks and then stored in 2%

phenoxyethanol for long-term preservation. Images were acquired using a ToupCam XCAM full HD camera connected to an Olympus microscope (model SZ61). Anatomical terminology of the skull and orbital tissue was determined according to the Nomina Anatomica Veterinaria (2017).

For scanning electron microscopy (SEM) experiments , small pieces of eyes were cut and fixed directly in 5% glutaraldehyde in a cacodylate buffer for 48 hours at 4°C. Samples were washed in three changes of 0.1% cacodylate buffer, post-fixed in a cacodylate-buffered solution of 1% osmium tetroxide for 2 hours at 37°C, and then washed three times in the same buffer. The specimens were dehydrated through an ascending series of ethyl alcohols and infiltrated with amyl acetate for two days. Drying was accomplished by critical point drying using liquid carbon dioxide. Specimens were mounted, sputter-coated with gold, and then examined on a JEOL scanning electron microscope (JSM-5400IV) at 15 kV\RL.

For light microscopy experiments , the lower jaws were removed from the samples, and heads were cut longitudinally into two halves. Specimens were preserved in 10% neutral formalin for three days, decalcified in EDTA for two weeks, dehydrated in a series of ethyl alcohols, cleared in methyl benzoate for three days, embedded in paraffin wax, and serially sectioned at 7 µm. Sections were stained with hematoxylin, eosin, and Masson's trichromic stains (Drury and Wallington, 1980). Images were acquired using an Olympus camera (model DP74) connected to an Olympus microscope (model BX43).

For transmission electron microscopy (TEM) investigation , small pieces of eyes (1 mm each) were cut and fixed in a cacodylate-buffered solution of 5% glutaraldehyde for 2 hours. Specimens were washed several times in the same buffer for 1 hour at pH = 7.2. Subsequently, the specimens were post-fixed in a cacodylate-buffered 1% osmium tetroxide solution for 2 hours at 4°C. Specimens were washed several times, as in the second step, followed by dehydration in a graded series of alcohol. The specimens were embedded in epoxy resin, treated for semi-thin sectioning at 1 µm thickness, and stained with toluidine blue for light microscopic examination.

Results

Anatomical and scanning electron microscopic investigations of the eyelids of Egyptian agama

The eye of the Egyptian agama, *Trapelus mutabilis* , has three eyelids: mobile upper and lower eyelids and a small, immobile nictitating membrane. The upper eyelid is shorter than the lower eyelid. The skin of the head above the upper eyelid extends laterally to form a superior extension. This extension bears large, plate-like scales that contain skin sense organs at their terminal points (Figs. 1a, b, and c). Highly variable polygonal scales are observed on the external surface of both eyelids, with approximately ten scales at the edges of the upper and lower eyelids, clearly observed by SEM (Figs. 1 and 2). The orientation of scales on the upper eyelid is similar to that on the lower eyelid; these scales are arranged in an imbricate order, and their surface features micro-ornamentation and Oberhäutchen with honeycomb-like structures. These honeycomb-like structures consist of numerous tiny honeycombs. Additionally, it was observed that the tips of these scales possess skin sense organs (Figs. 1d and 2b)\RL.

The anatomical investigation of the nictitating membrane of *T. mutabilis* revealed it to be a concave and non-mobile membrane. It is reduced and appears as a small projection connecting with the lacrimal gland posteriorly in the anterior canthus of the eye (Fig. 5a). SEM investigation of the nictitating membrane revealed that its surface has many folds marked by crypt-like structures (Fig. 5b).

Histological investigation of the eyelids of Egyptian agama

Histological investigation of the upper and lower eyelids of *T .mutabilis* revealed that the histological structure of both eyelids' external and internal surfaces is similar (Figs. 3 and 4)\RL.

The histological structure of the upper and lower eyelids and the superior extension of *T . mutabilis* revealed that the superior extension and the external surface of the upper eyelid are composed of keratinized stratified squamous epithelium with 2-4 nucleated cell layers, covered with a hard keratin layer forming the horny epidermal scales. The horny scales are connected by hinge regions (Figs. 3 and 4). The internal surface of the upper eyelid is composed of stratified cuboidal epithelium with 2-5 nucleated cell layers with oval and rounded nuclei. The superficial cells have small protrusions (Figs. 4C1 and C2)\RL.

The dermal layer (stroma) located between the external and internal surfaces is formed of fibrous connective tissue filled with densely packed collagen fibers of varying orientations, blood vessels, muscle fibers, and two types of pigment cells: iridophores and melanophores. Iridophores contain brownish-yellow granules and are located beneath the basement membrane of the keratinized stratified squamous epithelium. Melanophores appear as black cells with dendrite-like extensions scattered within the dermis (Figs. 3 and 4)\RL.

Histological investigation of the nictitating membrane of *T .mutabilis* revealed that it has two surfaces: an external surface (upper surface) and an internal surface (basal surface) (Fig. 5). The external surface of the nictitating membrane is composed of stratified squamous epithelium. In contrast, the internal surface comprises one or two nucleated cell layers with rounded nuclei connected to the conjunctival epithelium. The connective tissue stroma of the nictitating membrane is mainly made up of collagen fibers running in transverse directions and containing blood vessels (Fig. 5d). The conjunctival epithelium consists of one to three nucleated layers, and the connective tissue comprises collagen fibers, blood vessels, and hyaline cartilage (Fig. 5e).

Anatomical investigation of the ocular muscles of Egyptian agama

The ocular muscles include extraocular and accessory ocular muscles (Figs. 6 and 7).

A-Extraocular muscles

Dorsalis rectus muscle (Drm) \RL

The dorsalis rectus muscle is a sheet-like muscle composed of parallel fibers that run dorsal to the dorsalis oblique muscle and ventral to the medialis rectus muscle. It originates from the orbitosphenoid bone with fleshy fibers, spreading out to form two aponeurotic branches that insert on the scleral cartilage and the superior extension of the upper eyelid. This aponeurosis passes over the dorsal surface of the aponeurosis of the dorsalis oblique muscle. The origin of the dorsalis rectus muscle lies dorsal to the optic nerve, posterodorsal to the origin of the ventralis rectus muscle, and anterodorsal to the origin of the lateralis rectus muscle. The insertion site of the dorsalis rectus muscle is dorsal to the insertion site of the dorsalis oblique muscle\RL.

Origin: Fleshy, from the orbitosphenoid bone\RL.

Insertion: Aponeurotic, on the scleral cartilage and superior extension of the upper eyelid\RL.

Function: Elevation of the eyeball, upper eyelid elevation to help open the palpebral fissure, and extorsion.

Dorsalis oblique muscle (Dom)

The dorsalis oblique muscle is a thin, wing-shaped muscle formed of oblique fibers that run dorsal to the medialis rectus muscle and ventral to the dorsalis rectus muscle. It originates from the parabasisphenoid bone with fleshy fibers, then fans out obliquely to form two aponeurotic branches that insert on the scleral cartilage and the superior extension of the upper eyelid. The origin of the dorsalis oblique muscle is located posterodorsally to the origin of the ventralis oblique muscle. The insertion site of the dorsalis oblique muscle is ventral to the insertion site of the dorsalis rectus muscle\RL.

Origin: Fleshy, from the parabasisphenoid bone\RL.

Insertion: Aponeurotic, on the scleral cartilage and superior extension of the upper eyelid\RL.

Function: Elevation of the eyeball, elevation of the upper eyelid, and intorsion.

Medialis rectus muscle ***(Mrm)***

The medialis rectus muscle is a sheet-like muscle composed of thin parallel fibers located at the nasal pole of the eyeball, entirely covered by the dorsalis oblique muscle and the Harderian gland. It originates from fleshy fibers of the orbitosphenoid bone. These muscle fibers turn obliquely towards the medial surface of the eyeball to insert into the scleral cartilage, which is positioned inferior to the Harderian gland\RL.

Origin: Fleshly, from the orbitosphenoid bone\RL.

Insertion: Aponeurotic, on the scleral cartilage\RL.

Function: Pulls the eyeball towards the anterior nasal pole (adduction).

Ventralis rectus muscle ***(Vrm)***

composed of parallel fibers that run ventrally to the depressor palpebrae inferioris muscle. This muscle originates from fleshy fibers of the parabasisphenoid bone, fanning out to insert as aponeurosis on the scleral cartilage. Its insertion site is ventral to the insertion site of the ventralis oblique muscle on the sclera cartilage. The origin of the ventralis rectus muscle is located ventral to the optic nerve and anterodorsal to the origin of the lateralis rectus muscle\RL.

Origin: Fleshly, from the parabasisphenoid bone\RL.

Insertion: Aponeurotic, on the scleral cartilage\RL.

Function: Depresses the eyeball and causes rotation extorsion.

Ventralis oblique muscle ***(Vom)***

The ventralis oblique muscle is a sheet-like muscle with oblique fibers that runs ventrally to the depressor palpebrae inferioris muscle. This muscle is situated on the anteroventral surface of the eyeball in the nasal pole direction. It originates from fleshy fibers of the parabasisphenoid bone, fanning out to insert as aponeurosis on the scleral cartilage. The aponeurosis of the ventralis oblique muscle passes over the dorsal surface of the aponeurosis of the ventralis rectus muscle. Its insertion site is dorsal to the insertion site of the ventralis rectus muscle on the scleral cartilage. The origin of ventralis oblique muscle is located ventral to the optic nerve and anteroventral to the origin of the dorsalis oblique muscle\RL.

Origin: Fleshly, from the parabasisphenoid bone\RL.

Insertion: Aponeurotic, on the scleral cartilage\RL.

Function: Depresses the eyeball and causes rotation (intorsion/extorsion).

Lateralis rectus muscle ***(Lrm)***

The lateralis rectus muscle is a rectangular sheet muscle composed of oblique muscle fibers. It originates from fleshy, narrow fibers of the parabasisphenoid bone and extends to insert into the scleral cartilage. The origin site of the lateralis rectus muscle is ventral to the optic nerve and posteroventral to the origin site of the ventral rectus muscle. Its insertion site is dorsal to the insertion site of the bursalis muscle on the scleral cartilage\RL.

Origin: Fleshly, from the parabasisphenoid bone\RL.

Insertion: Aponeurotic, on the scleral cartilage\RL.

Function: Pulls the eyeball towards the posterior temporal pole (abduction).

Retractor bulbi muscle ***(Rbm)***

The retractor bulbi muscle is a sheet-like muscle composed of parallel fibers that run ventrally to the ventralis rectus muscle. It originates from fleshy, narrow fibers of the parabasisphenoid bone, spreading out to form a broad aponeurosis that inserts on the scleral cartilage. The origin site of the retractor bulbi muscle is ventral to the optic nerve and anterior to the origin site of the bursalis muscle. Its insertion site is ventral to the insertion site of the ventralis rectus muscle on the scleral cartilage\RL.

Origin: Fleshly, from the parabasisphenoid bone\RL.

Insertion: Aponeurotic, on the scleral cartilage\RL.

Function: Pulls the eyeball inward and outward within the socket.

Bursalis muscle (Bm)

fibers, appearing as a derivative of the retractor bulbi muscle. It originates from fleshy, narrow fibers of the parabasisphenoid bone, spreading out to form an aponeurosis that inserts into the scleral cartilage. The origin site of the bursalis muscle is ventral to the optic nerve and posterior to the origin site of the retractor bulbi muscle. Its insertion site is ventral to the insertion site of the lateralis rectus muscle. The tendon of the bursalis muscle runs anterodorsally toward the upper eyelid and anteroventrally toward the lower eyelid\RL.

Origin: Fleshly, from the parabasisphenoid bone\RL.

Insertion: Aponeurotic, on the scleral cartilage\RL.

Function: Pulls the eyeball inward and outward within the socket.

B- Accessory ocular muscles

Depressor palpebral inferioris muscle (Dpim)

The depressor palpebrae inferioris muscle is formed of tiny muscle fibers scattered within the periorbital fascia. It runs dorsally, covering the ventralis rectus muscle and ventralis oblique muscle. This muscle extends from its origin on the fleshly parabasisphenoid bone, fanning out towards its insertion on the connective tissue of the lower eyelid\RL.

Origin: Fleshly, from the parabasisphenoid bone\RL.

Insertion: Aponeurotic, on the connective tissue of the lower eyelid\RL.

Function: Contraction of the depressor muscle pulls the lower eyelid, resulting in the opening of the palpebral fissure.

Orbicularis oculi muscle

The orbicularis oculi muscle is not visible during the dissection of the eye of the Egyptian agama, *T. mutabilis* . Its fibers are located within the dermal layer of the eyelid stroma, adjacent to the internal surface of the eyelids. The orbicularis oculi muscle's contraction results in the palpebral fissure's closure.

Analysis of video-record of the action of the eye's muscles of Egyptian agama

The eye's motion was monitored via video recording in the laboratory (Fig. 8). Several video recordings were analyzed to identify movements, such as the opening and closure of the palpebral fissure and the elevation and depression of the upper and lower eyelids. However, identifying eyeball movements like elevation, depression, and rotation was challenging. The synchronization of eyelid movement with eyeball movement helped to observe these movements more clearly. This synchronization was particularly evident when the gaze was directed up or down or when the eye was elevated or depressed, resulting in significant movements of the eyelids\RL.

The elevation of the upper eyelid of *T. mutabilis* upward is performed by the contraction of the dorsalis rectus muscle, cooperating with the dorsalis oblique muscle, which elevates the eyeball. Conversely, the depression of the lower eyelid downward is performed by the contraction of the depressor palpebral inferioris muscle.

Simultaneous contraction of these upper and lower eyelid muscles expands the eye-opening, opposing the action of the orbicularis oculi muscle responsible for closing the eyelids.

Observations showed that when the pupil moves anteriorly with a decrease in skin surface at the nasal canthus, it indicates rotation of the eyeball towards the anterior direction, performed by the contraction of the medialis rectus muscle. Conversely, when the eyeball abducts away from the nasal canthus, it is performed by contraction of the lateralis rectus muscle.

Depression of the eyeball is achieved by the contraction of the ventralis rectus muscle and ventralis oblique muscle, while the elevation of the eyeball is performed by the dorsalis rectus and dorsalis oblique muscles.

A muscle never acts alone; in each antagonistic pair (lateralis-medialis, dorsalis-ventralis, and obliques), one muscle contracts while its companion relaxes. Most movements involve the simultaneous action of these three pairs. Additionally, observations indicated that the eyeball is retracted further into the eye socket by the retractor bulbi and bursalis muscles.

Discussion

The eyelids serve as integumentary duplications or folds of skin around the eyes, acting as a physical barrier in terrestrial vertebrates to protect against mechanical damage and desiccation while regulating light passage through the pupil (Johnson, 1927; Walls, 1942). Studies by Yasui et al. (2006) have confirmed that eyelids protect the eyeball and maintain a moist surface\RL.

The anatomical structure of the upper eyelid of *T. mutabilis* resembles that of the lower eyelid. However, the lower eyelid in this agama species is more mobile and can move downward through the contraction of the depressor palpebrae inferioris muscle, attaching to its base. Additionally, the lower eyelid appears larger than the upper eyelid, a common trait observed in reptiles and birds compared to mammals, where the upper eyelid is typically more mobile and larger than the lower one (Wyneken, 2012; Klećkowska-Nawrot et al., 2019)\RL.

Eyelids are movable structures supported by a connective tissue plate called the tarsal plate or tarsus (tarsus palpebralis). In most taxa, including lizards, birds, and mammals, the tarsal plate is described as a dense fibrous connective tissue, possibly containing cartilage, found within one or both of the upper and lower eyelids (Rieppel, 2000). Wyneken (2012) noted the presence of a tarsus in the eyelid of most lizards and crocodilians, with a bony structure in the upper eyelid of crocodilians and a cartilaginous structure in the lower eyelid of most lizards. However, turtles and tortoises lack these structures. In birds, the tarsal plate is located only in the lower eyelid and consists of dense connective tissue with elastic fibers (Klećkowska-Nawrot et al., 2016). In mammals, the tarsal plate is present in the upper and lower eyelids, comprising dense fibrous connective tissue (Klećkowska-Nawrot et al., 2019).

The eyelids of reptiles are covered by epidermal scales, displaying various patterns, shapes, thicknesses, and degrees of overlap among different species (Guerra-Fuentes et al., 2014; Wegener et al., 2014; Toni et al., 2007; Calsbeek et al., 2006). In *T. mutabilis* , the external surface of both upper and lower eyelids features highly polygonal scales of varying sizes and forms. The scales on the superior extension are notably large and plate-like acting as an umbrella for the eyes, serving as heat exchangers and aiding in water conservation in arid environments\RL.

Integument sense organs were first described in reptiles by Leydig (1868) in the lizard genera *Lacerta* and *Angus* and the snake genus *Coronella* . He identified small depressions on the surface of their scales, referred to as "organs of the 6th sense," and compared them to the taste buds of fishes and amphibians. Scortecci (1941) examined these receptors in detail in agamids, iguanids, and other lizards, distinguishing two principal types based on their structure: the first type has the Oberhäutchen with a bristle (or seta, or hair), while the second lacks bristles. The first type is typical of the integument of pygopodids, agamids, and gekkonids (Landmann, 1975)\RL.

The sense organs with the Oberhäutchen and bristle appear on the tips of some scales of the Egyptian agama's eyelids. These organs may serve as points of contact with the external environment. Maclean (1980) and Sherbrooke and Nagle (1996) suggested that these sense organs might work in conjunction with visual and other sensory systems to detect, locate, and apprehend prey, as noted by Barrett et al. (1970) in Crotaline and Boid snakes\RL.

The present study reveals that the structure of the two eyelids in *T. mutabilis* is very similar. The outer skin surface of the eyelid consists of two to four nucleated cell layers of keratinized stratified squamous epithelium, while the internal surface comprises two to five nucleated cell layers of stratified squamous epithelium.

Additionally, the distribution of pigment cells shows significant variability within the dermal layer, with large black melanophores (light-absorbing pigment) and dendrites that invaginate into the stratum basal of the upper and lower eyelids. These melanophores increase in number within the superior extension of the upper eyelid. Brownish-yellow iridophores (reflecting pigments) are scattered in large numbers within the dermis of the upper eyelid, its extension, and the lower eyelid. Saenko et al. (2013) and Teyssier et al. (2015) pointed out that these types of pigmented cells (iridophores) in reptiles participate in skin coloration and thermoregulation.

The third eyelid, known as the nictitans eyelid or nictitating membrane, is a prominent semilunar fold of the conjunctiva in Elasmobranchii fish, amphibians, reptiles, birds, and mammals (except humans) (Klećkowska-Nawrot et al., 2019). The nictitating membrane is sometimes lost in various taxa due to ecological differences, such as in whales, echidnas, opossums, marsupial moles, largely burrowing squamates like pygopodids, amphisbaenians, and dibamids, as well as chameleons and snakes (Caprette et al., 2004)\RL.

According to Schramm et al. (1994), the size of the nictitating membrane is inversely proportional to an animal's ability to remove foreign bodies from its eyes. This membrane, known as the haw, is well developed in ungulate mammals such as horses and cows but relatively small in carnivores like canines and felines. Cats can efficiently remove foreign bodies from their eyes using their paws. In aquatic animals, such as fishes and some amphibians, the anterior portion of the eye is cleaned by surrounding water, making the nictitating membrane unnecessary. The most prominent nictitating membranes are found in reptiles and birds exposed to wind, storms, dust, and sand (Schramm et al., 1994). A well-developed nictitating membrane is essential for keeping the corneal portion of the eye clean in species that cannot do so otherwise. In lizards, a well-developed nictitating membrane, associated with nictitating cartilages, completely covers the cornea, as seen in*Colobosaura modesta* and *Tretioscincus oriximinensis* (Guerra-Fuentes et al., 2014). Conversely, this membrane is reduced to a small projection at the base of the cornea and eyelid in*Alopoglossus angulatus* , *Cercosaura ocellata* , and*Micrablepharus maximiliani* (Guerra-Fuentes et al., 2014), similar to *T. mutabilis* . Rehorek et al. (2000) reported that reptilian species with a spectacle have lost the nictitating membrane, as in*Nothobachia ablephara* and *Calyptommatus sinebrachiatus* \RL.

The nictitating membrane in squamates is a movable fold that slides over the cornea (Walls, 1942). The exocrine secretion of the Harderian gland, attached to the medial region of the orbit (Guerra-Fuentes et al., 2014), lubricates this sliding movement (Payne, 1994). Oria et al. (2015) stated that the spreading of the tear film (orbital secretion) is accomplished by nictitating membrane movement rather than eyelid movement.

Crocodiles possess a well-developed nictitating membrane that moves obliquely backwards and slightly upwards. These animals often move the nictitating membrane across the eye without closing their eyelids. When closing the eye, the lower lid closes after the nictitating membrane has moved across the eye, unlike most other reptiles, where the membrane and eyelids move simultaneously (Johnson, 1927). In anura, the nictitating membrane moves from below upwards rather than horizontally or obliquely from the inner angle across the eye, as seen in all birds and many mammals (Johnson, 1927). Stibbe (1928) noted that birds require the fastest method for this movement, while mammals prioritize thoroughness over speed\RL.

Hiller (1995) reported that the conjunctival outer morphology of agamid species is mainly characterized by epithelial folds running parallel to the eyelid margins. These folds are present in all agamid species and contain scattered goblet cells that release secretory products onto the conjunctival surface. The goblet cell content in *T. mutabilis* is mucus, similar to the iguanid conjunctiva that mentioned by Hiller (1995)\RL.

Goodrich (1988) stated that extraocular muscles, responsible for eyeball movement, are consistent in number, arrangement, and innervation across vertebrates. El Hassni et al. (2000) noted that chameleons and higher vertebrates use the same musculature, but the performance of each type of movement varies according to lifestyle. Reptiles have six extrinsic eye muscles (medial rectus, lateral rectus, superior rectus, inferior rectus, inferior oblique, and superior oblique) responsible for rotating the eyeball within the orbit (Kardong, 2012). These muscles are characterized by fine movement and coordinated control of the eyes with the vestibular system (Kardong, 2012; Schwab, 2012). Eye movement is essential to prevent photoreceptor fatigue (Schwab, 2012). Turtles, crocodilians, and most lizards (except Heloderma) have mobile eyes, while snake eyes are immobile (Underwood, 1970).

The dorsalis rectus muscle and dorsalis oblique muscle in *T. mutabilis* are divided into two parts with different origin sites on the orbitosphenoid and parabasisphenoid bones but share the same insertion in the sclera and upper eyelid. The eye of *T. mutabilis* lacks levator muscles (levator palpebrae superioris), so the dorsalis rectus muscle and dorsalis oblique muscle are responsible for elevating the upper eyelid.

In reptiles, the protective reflex caused by the retraction of the eyeball in response to corneal stimulation (Oelrich, 1956; Tansley, 1965) seems to be accomplished by simultaneous contractions of two retractor muscles, the retractor bulbi and bursalis, as observed in the lizard *Varanus* (Barbas-Henry and Lohman, 1988; El Hassni et al., 2000). Retractor oculi and protractor oculi, known as the levator bulbi or retractor bulbi muscle in reptiles, are inserted on the sclera just adjacent to the optic nerve and are responsible for inward and outward eye movement within the socket (Wyneken, 2012). The present results showed that the retractor bulbi and bursalis muscles of *T. mutabilis* are inserted on the scleral cartilage, with their origin sites lying ventral to the optic nerve. These muscles pull the eyeball inward and outward within the socket.

Retractor bulbi muscles are synapomorphic for tetrapods and are subsequently lost in snakes and birds. Losing these muscles in snakes results in a condition convergent with primitive aquatic animals. This loss implies visual reduction, similar to the loss observed in large-eyed forms with great visual acuity, like birds (Caprette et al., 2004).

Bour et al. (2000) stated that in the absence of retractor bulbi muscles, retraction is produced by the simultaneous contraction of multiple extraocular muscles. In some reptiles, the retractor bulbi is assisted by one of two additional muscles: the quadratus (in lizards) or the pyramidialis (in crocodiles and turtles). In birds, the globe of the eye fits so tightly into the eye socket that little room is left for retraction. Therefore, the retractor bulbi is not present and is replaced by both quadratus and pyramidalis muscles. These muscles slide the nictitating membrane over the cornea without retraction of the eye (Butler and Hodos, 2005).

In terrestrial mammals, well-developed retractor muscles are usually associated with the presence of a nictitating membrane (Duke-Elder, 1958; Spencer and Porter, 2006). Retractors are absent in some mammals, such as the aardvark, flying fox, and primates. In mammals, the retractor bulbi can be divided into two (mouse), three (dog), or four (cat) muscle bellies (Matheus et al., 1995; Meshida et al., 2020).

In conclusion, all these characteristics that mentioned above are well-suited for the remote desert environment in which the agama lives.

Data availability statement

All data sets generated and utilized in this study can be found in the manuscript and Supporting Information.

Funding statement

Not applicable

Conflict of interest disclosure

The authors declare no conflict of interest.

Ethics approval statement

The study was approved by the guidelines of the Research Ethics Committee, Assiut University

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Figure Legends

Fig.1. Photomacrograph of the eye of Egyptian agama, *Trapelus mutabilis* (a) and Scanning electron micrograph (b, c, d) showing the upper eyelid (Ue) with superior extension (star), lower eyelid (Le), polygonal scales (arrow), skin sense organ (white arrowhead), and micro-ornamentation of honeycomb-like scales (black arrowhead).

Fig.2. Scanning electron micrograph of the upper and lower eyelid of Egyptian agama, *Trapelus mutabilis* showing (a, b, c) the micro-ornamentation of honeycomb-like scales (black arrowhead) wit skin sense organ (white arrowhead).

Fig.3. Photomicrograph of eyelids of Egyptian agama, *Trapelus mutabilis* , showing (A) complete cross section of the Egyptian agama's eye; (U1, U2, U3) the structure of the upper eyelid and (S1, S2) its superior extension;

Fig.4. Photomicrograph of eyelids of Egyptian agama, *Trapelus mutabilis* , showing (A) complete cross section of the Egyptian agama's eye; (L1, L2, L3) the structure of the lower eyelid and (C1, C2) conjunctiva surface.

Fig.5. Photomicrograph of nictitating membrane of Egyptian agama, *Trapelus mutabilis* , showing; (a) the location of the small third eyelid (Nm) related to lacrimal (Lg) and harderian gland (Hg) and sclera ossicles (So); (b) scanning electron micrograph of the surface of nictitating membrane (Nm) has many folds marked by crypt-like structures, and pores of lacrimal gland (zigzag arrow); (c, d,e) photomicrograph of the transverse section of the nictitating membrane showing external surface (E) and internal surface (I) and its cartilaginous support (Ce)

Fig.6. Photomacrograph of the eye's dissection of Egyptian agama, *Trapelus mutabilis* , after removing the skin of the upper eyelid to show the dorsalis rectus muscle (Drm), dorsalis oblique muscle (Dom) and depressor palpebral inferioris muscle (Dpim).

Fig.7. Photomacrograph of the eye's dissection of Egyptian agama, *Trapelus mutabilis* , after cutting the dorsalis rectus muscle (Drm), dorsalis oblique muscle (Dom), depressor palpebral inferioris muscle (Dpim) to show the medialis rectus muscle (Mrm), tendon of bursalis muscle (star) and position harderian gland (Hg),

ventralis rectus muscle (Vrm), ventralis oblique muscle (Vom), lateralis rectus muscle (Lrm), harderian gland (Hg), retractor bulbi muscle (Rbm), tendon of bursalis muscle (star), lower eyelid (Le), and the originate sites of these muscle on eyeball and interorbiral septusm.

Fig.8. Photo of eye of Egyptian agama, *Trapelus mutabilis* , showing the different actions; the elevation and depression of upper and lower eyelid respectively (opening phase) (black arrow); the down action of upper (green arrow) and up action of lower eyelid (red arrow) via the contraction the orbicularis oculi muscle (closure phase); the adduction and rotation of the eye ball toward the nasal canthus (blue arrow) via contraction of medialis rectus muscle; the abduction and rotation of the eye ball toward the nasal canthus (yellow arrow) via contraction of lateralis rectus muscle; the depression action of eyeball via contraction of ventralis rectus and oblique muscle (white arrow); the elevation action of eyeball via contraction of dorsalis rectus and oblique muscle (orange arrow); and the action of retractor bulbi and bursalis muscle (star) yielding the pulling the eyeball inward the socket.

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`photo.docx` available at https://authorea.com/users/796610/articles/1227307-anatomical-scanning-electron-microscopic-and-histological-studies-of-the-ocular-lids-and-muscles-of-the-egyptian-agama-trapelus-mutabilis