



# Microstructure Adaptation in Mammalian Cornea a Comparative Study

ZEINAB ABDELTAH, AHMED RAGAB, RASHA E. ABO-ELENEEN, AHLAM M. EL-BAKRY\*

Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

**Abstract** | Due to the epithelial ion barrier, the stromal latticelike structure of collagen fibrils, and the endothelial cells, the corneas of most vertebrate's species stay transparent. The corneal epithelium, the corneal endothelium, or both, play an important role in controlling corneal hydration, depending on the species. Three mammalian families [Pteropodidae (*Rousettus egyptiacus*), Leporidae (*Oryctolagus caniculus*), Muridae (*Rattus norvegicus*)] of the present study were collected from different locations of Egypt. The investigated cornea was dissected free from the orbit and prepared for light and scanning electron microscopy and special stain. Three investigated mammalian species composed of three identical layers; epithelium, stroma, and endothelium besides appearance of Bowman's membrane in *Rousettus egyptiacus* and *Oryctolagus caniculus*. On the other hand, Descemet's membrane appear only in *Oryctolagus caniculus*. The investigated epithelial layers showed moderate affinity to stain with by Periodic Acid Schiff stain in *Rousettus egyptiacus* and *Oryctolagus caniculus*, although high activity with PAS stain in *Rattus norvegicus*. Their corneal epithelium cells distinguished by irregular polygonal cells covered with moderated density of shorted microplicae. The ultimate goal is to keep the cornea transparent and provide the greatest refract. According to the rabbit cornea and its adaptive trends have eloquently described the vertebrate eye's relative optical features. The study presents a brief image/account of certain structures of cornea for three of mammalian's species and to correlate them with the different circumstances and variation of habitats.

**Keywords** | Cornea, Mammals, SEM, Stroma, Microprojections

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\***Correspondence** | Ahlam M. EL-Bakry, Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt; **Email:** amalbakry2@yahoo.com

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## INTRODUCTION

Optically, the cornea is considered the eye's transparent, the anterior layer protecting the eye and refracting light. It is coated with a tear film that offers nearly two-thirds of the refractive power of the eye together with the underlying tissue (Nejad *et al.*, 2015). The cornea is made up of five layers in many mammals. Whereas their arrangement from the outer to inner is the epithelium, Bowman's membrane, the stroma, Descemet's membrane, and the endothelium (DelMonte and Kim, 2011).

Considering, the epithelium is the cornea's outermost layer with regeneration capability after an injury. Furthermore, the endothelium is made up of non-renewable cells that maintain the dehydrated stroma and transparent cornea.

The endothelium's permeability can influence the cornea's transparency and capacity to work properly (Iannaccone *et al.*, 2012). The stromal collagen fibril arrangements play a significant part in cornea's mechanical strength. Throughout the lamellae, the permanent orientations of the collagen fibrils lead to corneal anisotropy (Sheppard *et al.*, 2010). In addition, the keratocytes connect to their adjacent cells by interconnecting fine cell procedures and constitute cellular networks (Murata *et al.*, 2019). Varieties in corneal epithelium thickness considered a structural adaptation occurring in different vertebrates and allowing them to adapt to a broad range of environmental circumstances (Edelhauser, 2006; Hutcheon *et al.*, 2019).

Many authors have shown in mammals that there are a decrease in corneal metabolism and decrease in the

glycogen deposition, besides adversely impacts the barrier characteristics and transport functions of both epithelium and endothelium, leading to an rise in corneal thickness and a loss of transparency (Edelhauser, 2006; Parekh et al., 2019). Metabolic power and glycogen energy are crucial for the maintenance of mammalian corneal border layers ' barrier function and ionic pumps, especially corneal endothelium. Furthermore, there are decrease in epithelial corneal density, therefore the endothelial cells develop tight junctions. Also, the endothelial permeability decreases, the number of endothelial cell pump sites and density increase, consequently the total corneal hydration decrease resulting in a transparent cornea (Edelhauser, 2006). Comparative morphological surveys have recognized a variety of animal eyes, including pinhole; mirror and single-lens camera eyes by several techniques (Land and Nilsson, 2012). On the other hand, there are little investigations on some species of mammals like bats. Besides, the present study aims to correlate between the significant effect of the environmental condition, and the morphological and anatomical structures of the cornea in the investigated species.

## MATERIALS AND METHODS

### EXPERIMENTAL ANIMALS

Three mammalian families of the present study were collected from different locations of Egypt and were identified according reviews of Mammalian (Saleh, 1997). Ten animals from each family were used in the present study. The animals were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infections and to acclimatize on the laboratory conditions. Their common name, distribution and their habitat were explained in the present investigation.

### FAMILY PTEROPODIDAE: *ROUSTTUS EGYPTIACUS* (E. GEOFFROY, 1810)

*Roustitus egyptiacus* (E. Geoffroy, 1810) has the common name as Egyptian fruit bat. They distributed in Behira Governorate, Egypt (30.8481° N, 30.3436° E) and Abo-Rwash (30.0131° N, 31.2089° E). They were found in moist tropical habitat and subtropical areas. They considered as nocturnal animals and spent their days roosting in trees or caves, often with large groups of other bats with weight ranged 980± 260 g (Mickleburgh et al., 1992).

### FAMILY LEPORIDAE: *ORYCTOLAGUS CANICULUS* (LINNAEUS, 1758)

*Oryctolagus caniculus* (Linnaeus, 1758) has the common name as the Domestic rabbit. They distributed Behira Governorate, Egypt (30.8481° N, 30.3436° E) and Abo-Rwash (30.0131° N, 31.2089° E). They preferred a mixed habitat of Mediterranean oak savanna, or area with 40% cover for shelter from predators. They considered

as herbivorous mammals and gregarious and readily domestics. They were being most active around dawn and dusk, although they weren't infrequently seen active during the day 1120±320g (Lombardi et al., 2003).

### FAMILY MURIDAE: *RATTUS NORVEGICUS* (BERKENHOUT, J. 1769)

*Rattus norvegicus* (Berkenhout, J. 1769) have common name as Albino rat. They distributed Behira Governorate, Egypt (30.8481° N, 30.3436° E) and Abo-Rwash (30.0131° N, 31.2089° E). They inhabited sandy and sometimes rocky areas and live in tunnels under shrubs with diurnal activity with weight ranged from 120±12.0g.

### EXPERIMENTAL METHODS

After enucleation the corneas were dissected free from the orbit with a sharp out razor blade and prepared for histological, histochemical and scanning electron architectures as follows:

### HISTOLOGICAL SECTIONS

The fixed specimens of the cornea were washed to remove the excess of the used fixative. They were then dehydrated in ascending grades of ethyl alcohol 70, 80, 90 and 95% for 45 minutes each, then in two changes of absolute ethyl alcohol each for 30 minutes. This step was followed by clearing in two changes of xylene, each for 30 minutes. The tissues were then impregnated with paraplast plus (three changes) at 60°C for three hours and then embedded in paraplast plus. Sections of 4 to 5 µm thickness were prepared with a microtome and stained with hematoxylin and eosin for histopathological examination (Suvarna and Layton 2013). After light examination for the corneal histological sections; the presence and absence of different layers were recorded as follows: absent (-); one layer (+); two layers (++) and three layers (+++).

### HISTOCHEMICAL SECTIONS BY PERIODIC ACID-SCHIFF'S (PASSO ET AL., 2019)

The fixed specimens de-waxed by xylene and rehydrated through graded ethanol to water. Then they oxidized with 1% aqueous periodic acid solution (H<sub>5</sub>IO<sub>6</sub>; WINLAB; Leicestershire, UK) for five minutes. After that, they were rinsed in distilled water and were covered with Schiff's reagent for 10-15 minutes then rinsed in running tap water for five minutes. Finally, the specimens were dehydrated, clear with xylene, mounting, and covered slips. The sections were ready for examining by light microscope (McManus, 1946; Bancroft et al. 2013). The degree of carbohydrates was evaluated and represented as - = absence; + = mild; ++ = moderate and +++ = high.

### SCANNING ELECTRON MICROSCOPY (SEM)

The whole eye was immediately fixed overnight with

modified Karnovsky solution (2% paraformaldehyde and 2.5% glutaraldehyde containing 0.1 M cacodylate-buffer, pH 7.4) according to Morris (1965). The fixed specimens were then washed in 0.1 M cacodylate buffer and post-fixed in a cacodylate-buffered solution of 1% osmium tetroxide at 37°C for 2 hours. The corneal specimens were rinsed in increasing concentrations of ethanol to commence dehydration which the first rinsed in 50 % ethanol for 5 minutes; then 3 times for 5 min with 70% ethanol; rinsed 3 times for 5 min with 90% ethanol, rinsed 2 times for 5 min in 100 % ethanol; and finally rinsed 2 times for 10 minute in 100% ethanol (molecular sieve) all ethanol was removed from the samples and replaced with hexamethyldisilane (HMDS) in fume hood and left for 10 minutes. The base of the metal SEM stubs was labeled and applied double-sided conductive carbon tape to the top of the stubs. The dried specimens were then sputtered with gold in Joel fine coat Ion Sputter (SPI-Module). Specimens were then examined and photographed using standard microscope operating procedures JEOL SEM (JSM-5400 LV) for visualization of specimens in the Microanalysis Centre, Faculty of Science, Beni-Suef University, Beni-Suef Egypt. The analysis was commenced at low magnification, then the magnification increased gradually at an accelerating voltage of 15kv (JSM.5400LV, JEOL) (Jeffree and Read 1991; Goldstein et al., 2017).

IMAGE ANALYSIS

The anterior surface of the investigated cornea of each studied species was examined by JEOL (JSM with accelerating voltage 5400 LV). The area of the individual epithelial cells (µm<sup>2</sup>) and the number of cells were measured (Abramoff et al., 2004). Moreover, the mean of epithelial cell density was calculated in addition to the diameters of both microholes and blebs. These image analyses were digitally recorded using Image J software.

STATISTICAL ANALYSIS

Data were analyzed using one- way analysis of variance (ANOVA). Mean for ten readings for the corneal surface for each parameter (microholes and blebs) at least for measuring. Data were expressed as mean ± standard deviation (SD). Values of P> 0.05 were considered statistically non-significant, while values of P< 0.05 were considered statistically significant.

ETHICS COMMITTEE APPROVAL

All animal procedures were conducted following the standards set in the guidelines for the care and use of experimental animals by the Animal Ethics Committee of the Zoology Department in the Faculty of Science at Beni-Suef University (under an approval number is BSU/FS/2015/9).

HISTOLOGICAL OBSERVATIONS

The cornea of *Roustitus egyptiacus*, *Oryctolagus canuculus*, and *Rattus norvigicus* are illustrated throughout Figure 1. They arranged as a superficial epithelial layer, followed by stroma and ended with the endothelium layer. Stromal lamellae were characterized by good distribution of keratocytes in its matrix. Besides, their cornea ended with monolayer of endothelium of flat squamous cells. Bowman’s membrane was illustrated in *R. egyptiacus* and *O. canuculus*, but absent in *R. norvigicus*. Descemet’s membrane appeared only in *O. canuculus*. The interfibrille space spread only in *R. egyptiacus*, *O. canuculus* among their collagen lamellae (Figure 1 and Table 1).

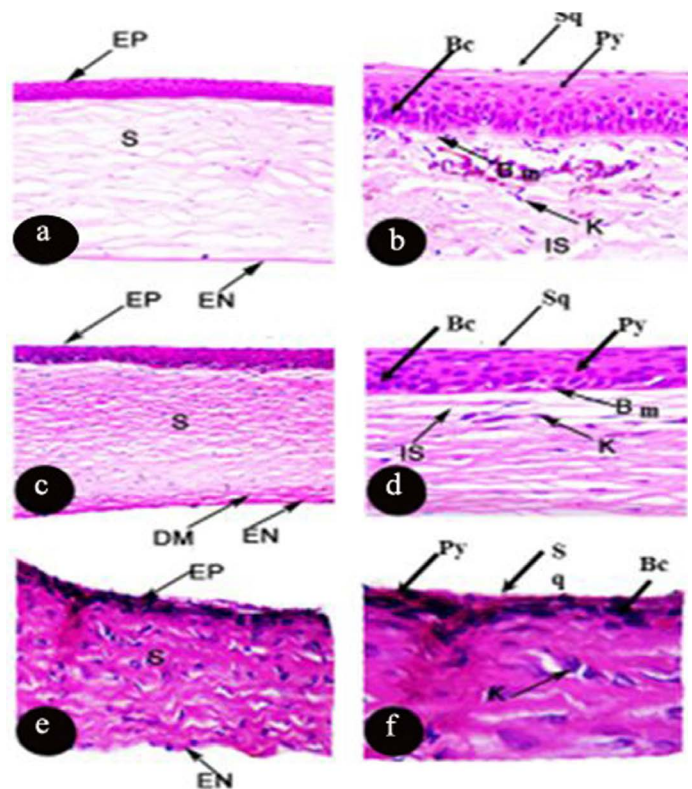


Figure 1: Photomicrograph of transverse section of the cornea of *Roustitus egyptiacus* (a, b) *Oryctolagus cunculus* (c, d), *Rattus norvegicus* (e, f). (a, c, e H and E X100). (b, d, f H and E X400).

Particularly, the epithelial layer of *Roustitus egyptiacus* was distinguished by one layer of basal columnar cells, followed by six rows of polyhedral cells and ended with one row of flat squamous cells. However, cornea of *O. canuculus*, their epithelium based on one row of cuboidal cells with round nucleus, four rows of polygonal cells and ended with one layer of flat squamous cells. On the other hand, *R. norvigicus* cornea was distinguished by a simple epithelial layer which arranged as one layer of basal cuboidal cells, then another one layer of polygonal cells and ended with flat squamous cells (Figure 1 and Table 1).



**Table 1:** Comparison between corneal layers and its histological structure in three species of mammals:

Layer	Epithelial layer (Types of Cells / Layers)				Bowman's membrane	Stroma	Descemet's membrane	Endothelium
	Basal columnar layer	Basal cuboidal layer	Polyhedral layers	Flat squamous layer				
<i>Rousettus egyptiacus</i>	+	-	+++++	+	+	+	-	+
<i>Oryctolagus caniculus</i>	-	+	++++	+	+	+	+	+
<i>Rattus norvigis</i>	-	+	+	+	-	+	-	+

**Table 2:** Comparison between the thicknesses (Mean ±SD) of corneal layers in three species of mammals (10 animals per species). Layer's thickness that are not sharing common superscripts denote significant differences (P< 0.05).

Species	Total thickness (µm)	Corneal Layers		
		Epithelium thickness (µm) (%)	Stroma thickness (µm) (%)	Endothelium thickness (µm) (%)
<i>Rousettus egyptiacus</i>	340.7±24.9 <sup>b</sup>	35.7±4.3 <sup>a</sup> (10.4%)	301.0±38.6 <sup>b</sup> (88.5%)	3.9±0.2 <sup>a</sup> (1.1%)
<i>Oryctolagus caniculus</i>	480.9±104.2 <sup>c</sup>	33.3±3.9 <sup>a</sup> (7%)	441.6±54.6 <sup>c</sup> (91.8%)	6.2±1.3 <sup>b</sup> (1.2%)
<i>Rattus norvigis</i>	281.0±46.6 <sup>a</sup>	37.2±8.4 <sup>a</sup> (13.4%)	221.2±20.6 <sup>a</sup> (78.7%)	26.4±4.8 <sup>c</sup> (9.3%)
F Value	34.1	2.6	42.8	339.5
P Value	0.000	0.08	0.000	0.0000

Obviously, the thickness of the mammalian epithelial cornea showed non-significant relation between the examined mammals' species (P<0.05) whereas, Table 2 recorded 35.7±4.3, 33.3±3.9 and 37.2±8.4 µm for *R. egyptiacus*, *O. caniculus* and *R. norvigis* respectively. Therefore, this data revealed that the mammalian epithelium constituted relatively 10% of corneal thickness.

Furthermore, the stromal thickness of the studied species apparently constituted around 86% from the total thickness. Table 2 showed a statistically significant relationship among the investigated species (P<0.05), which was recorded in *R. egyptiacus* and *O. caniculus* and *R. norvigis* 301.0±38.6, 441.6±54.6 and 221.2±20.6 µm respectively.

Moreover, the endothelial thickness was measured in *R. egyptiacus*, *O. caniculus* and *R. norvigis* 3.9±0.2µm, 6.2±1.3µm and 26.4±4.8 µm respectively and reflect a highly significant relationship among the investigated species (P>0.05). Table 2 revealed that the endothelium thickness registered around 1% from the total thickness except *R. norvigis's* endothelium, which reported relatively 9% from the whole thickness.

The current investigation revealed that the highest total corneal thickness and the stromal layer (91.8%) recording for *O. caniculus*. In contrast to *R. norvigis* has the higher epithelium and endothelium layers 13.4% and 9.3%, respectively.

**HISTOCHEMICAL OBSERVATIONS: (PERIODIC ACID SCHIFF (PAS) REACTION)**

Generally, the large storage of glycogen in corneal epithelium plays a vital role in the transparency function of cornea. Glycogen distributed among the corneal layers

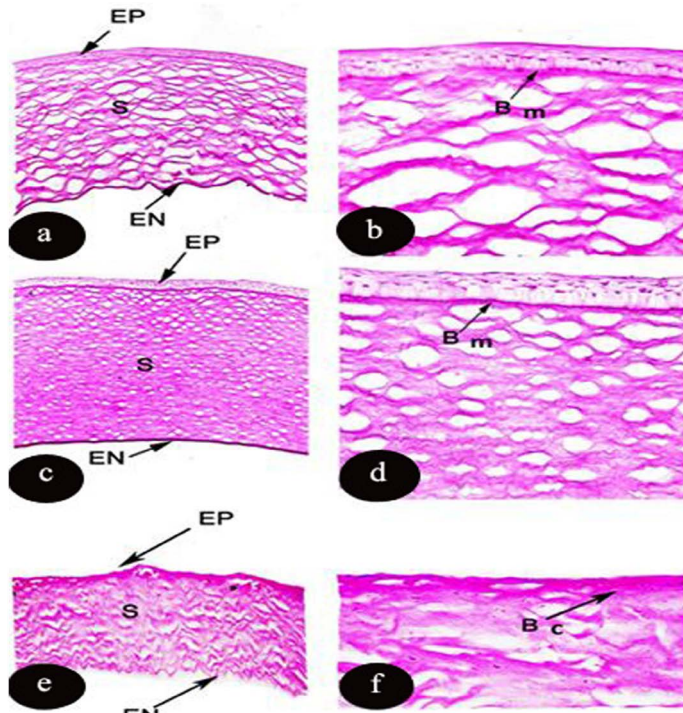
of the investigated vertebrate's species. Epithelium and endothelium are richer in glycogen than the stromal layers in some species. Bowman's and Descemet's membranes were represented in some species and others were not.

The epithelial layer of the mammalian cornea was intensely stained in *Rattus norvigis* however, it moderated in *Oryctolagus cunculus* and *Rousettus egyptiacus*. On the other hand, stroma and endothelium layer had strong PAS reaction in all studied species *Rousettus egyptiacus*, *Oryctolagus cunculus* and *Rattus norvigis*, respectively. Concerning Bowman's membrane had strong affinity to PAS materials in only two examined species (*R. egyptiacus* and *O. caniculus*). However, Descemet's membrane is completely absent from the three investigated species (Figure 2 and Table 3). It was noticed that the basal columnar of the epithelium possessed high affinity to PAS materials.

**SCANNING ELECTRON MICROSCOPY (SEM) OBSERVATIONS**

The mammalian central cornea distinguished by highly significance cell density, which *R. egyptiacus* had the highest value 8428.0±450.4cells per mm<sup>2</sup>, while *R. norvigis* recorded 2693.4±308.7cells per mm<sup>2</sup> and *O. caniculus* had 2402.9±178.8cells per mm<sup>2</sup> (Table 4).

Irregular polygonal epithelial cells were mostly predominantly in two mammalian investigated species *O. caniculus* and *R. norvigis*, although it was regular in shape in cornea of *R. egyptiacus*. Concerning, microplicae which considered as surface micro projection and were exposed by two types of polymorphic cell according to their scatter electron in each examined species which was light (L) and dark in *R. egyptiacus* and *O. caniculus*, however medium and light in *R. norvigis*.

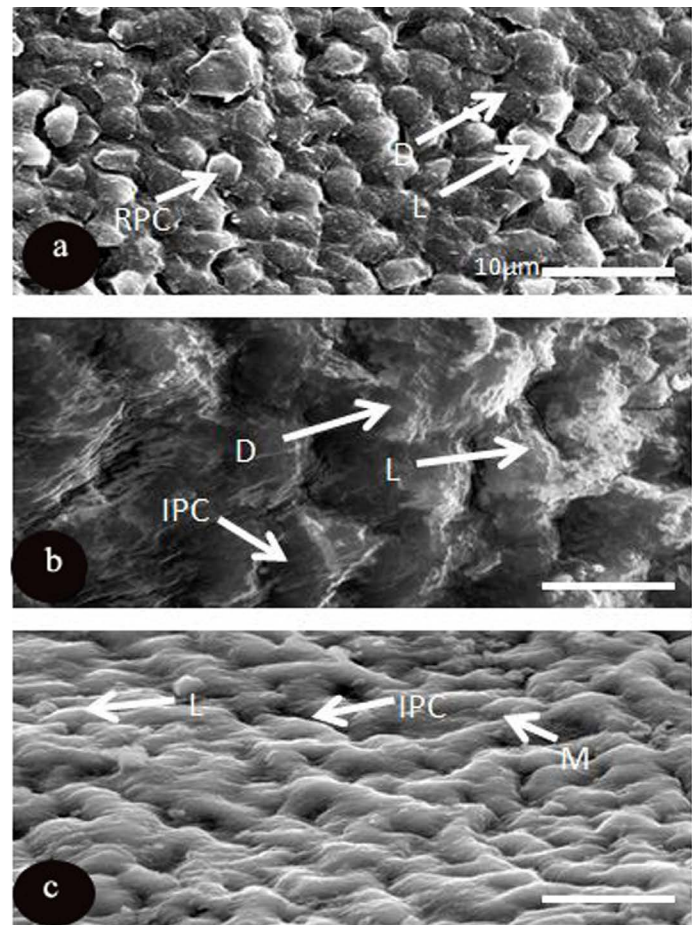


**Figure 2:** Light micrograph of transverse section of the cornea of *Rousttus egyptiacus* (a, b) *Oryctolagus cuniculus* (c, d), *Rattus norvegicus* (e, f). Showing PAS reaction in the corneal layers. (a, c, e): (PAS X100), (b, d, f): higher magnification (PAS X400).

The light type was represented by dense microplacae covering the surface of light cells, where the medium cells formed of semi dark cells covered with moderate density of microplacae, while dark ones consisted of dark epithelial cells, which had less density of microplacae (Figure 3). Microprojection was represented by various diameter over the corneal surface of *O. caniculus* and *R. norvigis*. In spite of blebs distributed on the surface of *R. egyptiacus* in round shapes and with different diameters  $5.8 \pm 1.6 \mu\text{m}$  (Figure 4 and Table 4).

Concerning the histological examination of the studied mammalian corneal sections referred to presence of five morphological layers; Defensor epithelium, mechanical barrier basement membrane Bowman's membrane, hypocellular stroma, bounded Descemet's membrane and integrated endothelium. Similarly, these observations are postulated (El-Dawi, 2005) in some mammalian species. The current results referred that the mammalian corneal

transverse sections composed of stratified squamous epithelial layer in the three investigated species. (Doughty, 1990; Brookes, 2018) confirm the same findings in rabbits and rats.



**Figure 3:** SEM micrograph of the corneal epithelial cell of three mammalian species a: *Rousttus egyptiacus* b: *Oryctolagus cuniculus*; c: *Rattus norvegicus*. (Scale bar,  $10 \mu\text{m}$ ).

Optically, it is noticed that the increase in number of epithelial layers protect the cornea from any chemical or mechanical abrasion and stress from the outside environment. Similar observations are suggested in *Rattus rattus* and *Meriones shawi* (El-Dawi, 2005) and in the Knockout mice (Doughty and Fong 1992; Chakravarti, 2001; Saadi-Brenkia et al., 2018). Unlike (Gibson et al., 1995; El-Bakry 2011) are in disagreement with our findings in some species of mammals.

**Table 3:** Comparison between three investigated mammalian species according to carbohydrate content in cornea by using Periodic Acid Schiff (PAS) stain: (+++) strong reaction; (++) moderate reaction; (+) weak reaction; (-) absent Periodic Acid Schiff (PAS) stain.

Mammalian	Epithelium	Bowman's membrane	Stroma	Descmet's membrane	Endothelium
<i>Rousttus egyptiacus</i>	++	+++	+++	-	+++
<i>Oryctolagus cuniculus</i>	++	+++	+++	-	+++
<i>Rattus norvigis</i>	+++	-	+++	-	+++



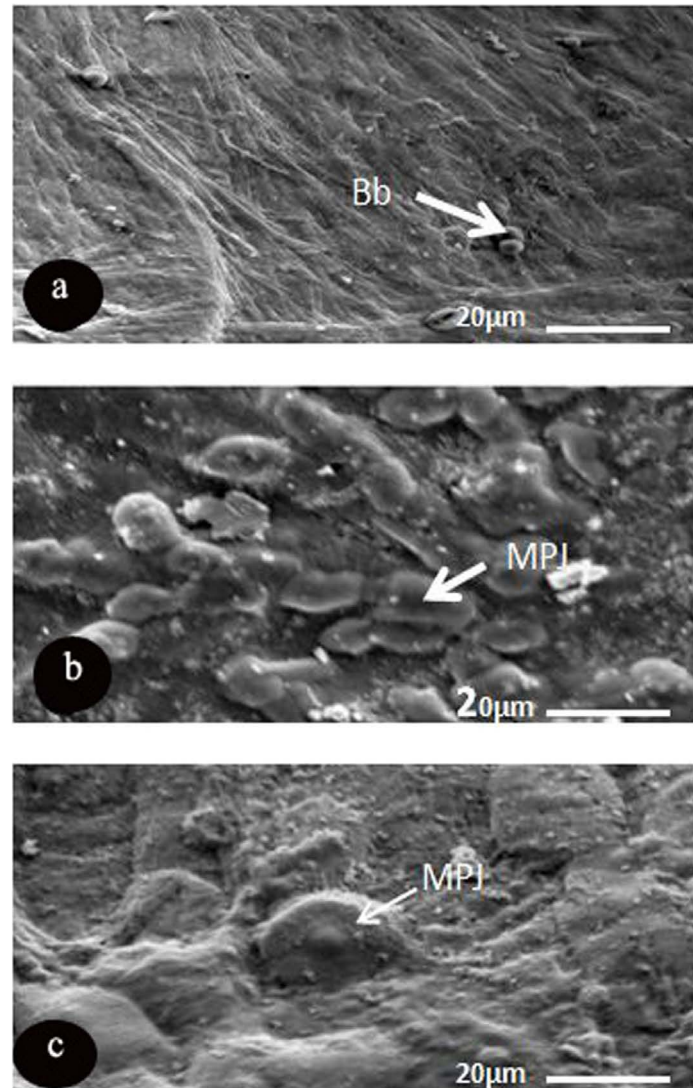
**Table 4:** Comparison between the cell density (Mean ±SD) of corneal epithelial layer and diameter of blebs and microholes in three mammalian species (10 animals per species). Measurements that not sharing common superscripts denote significant differences (P<0.05). NM: not measured. \* (NM), not measured. All measures of epithelial cell density are calculated from central cornea.

Class	Species	Epithelial cell density (cells/ mm <sup>2</sup> )	Blebs (µm)	Micro holes (µm)
Mammals	<i>Roustitus egyptiacus</i>	8428.0±450.4 <sup>b</sup>	5.8±1.6	NM*
	<i>Oryctolagus caniculus</i>	2402.9±178.8 <sup>a</sup>	NM*	NM*
	<i>Rattus norvegicus</i>	2693.4±308.7 <sup>a</sup>	NM*	NM*
	F value	629.2	NM*	NM*
	P	0.000	NM*	NM*

Accordingly, taking into consideration that ocular size affects the number of epithelial layers, as a response to adapt with the hard conditions of their terrestrial environments. These considerations probably confirmed the correlations between the optical size and increase in thickness of the epithelial layers thus, leading to the formation of an excellent focal system. That might be a monofocal system such as in *Canis lupus lupus* and *Canis lupus familiaris* or multifocal optical system as in *Mus musculus* (Malmström and Kröger 2006; Neagu and Petraru, 2015). The morphological function of the epithelial layer of the studied species reflects its physiological activities such as biochemical reaction, refraction power and accommodation with hard habitat. Variation of environmental agents among examined species like nocturnal vision in *Roustitus egyptiacus* and diurnal vision in *Oryctolagus caniculus* and *Rattus norvegicus* reflect the specialization of the morphological design of the nocturnal cornea and its strong curving to provide higher refractive index, that estimated entrance of more light to the retina for improving the transparency of cornea and adaptation with the nocturnal vision. These architectural designs are found in some nocturnal mammals (Neagu and Petraru, 2015)

Such forested habitat of *R. egyptiacus* rather than the other two investigated species, multi polygonal layers arranged in its epithelial layer as a mechanical defense and accommodation mechanism with nocturnal conditions against any environmental influences. Similar recordings is addressed in nocturnal species of *Bubo Bubo africans* (Pourelis, 2013).

Another characteristic structure in the examined mammalian corneal sections is uniformed distribution of condensed collagen fibrils. They arrange as one lamellar zone without interpretations and with relatively equal interfibrillar spaces. Such unique architecture is reported in some land mammals (Lemp et al., 1970; Leonard et al., 2016). *In vitro* studies of (Nagayasu et al., 2009; de Oliveira et al., 2018) refused our findings on other mammals such as dogs and also (El-Dawi, 2005) in *Rattus rattus* and *Meriones shawi*.



**Figure 4:** SEM micrograph of the corneal epithelial cell of three mammalian species showing. a: *Roustitus egyptiacus*; b: *Oryctolagus cuniculus*; c: *Rattus norvegicus*. Note: blebs (Bb) and microprojection (MPJ) (Scale bar, 20µm).

It is noticed that the collagen lamellae of nocturnal studied mammals (*R. egyptiacus*) have relatively large interfibrillar spaces than other two diurnal examined species (*O. caniculus* and *R. norvegicus*) which assist in the formation of a clear and sharp retinal image. As large interfibrillar spaces allow a large image to reach to the retina and enhancing

the corneal transparency (Meek and Knupp, 2015; Neagu and Petraru, 2015).

To date, many attempts to explain the correlations between the corneal transparency and the complexity of the collagen lamellae whereas the transparency increases with decreasing the parallelism and condensation of the stromal lamellae (Meek and Knupp, 2015). So, these findings are suggested in our investigated species, with relative appearance of the loose lamellae in *R. egyptiacus*, more condensed in *O. caniculus* and most condensed in *R. norvigus*.

As mentioned in the current histological examination, uniformed distribution and diameter of the collagen fibril of the stromal layer played a vital role in the corneal transparency (Meek and Knupp, 2015).

Excellent hexagonal lattice was constricted from interconnecting of the fibrils with each other, preserving distance between them. Similarly, the intensity of the entering light is inversely proportional with the radius of the fibrils. The present data confirmed these findings in the examined species, in which *R. egyptiacus* possessed the lower fibril radius and the higher interfibrillar space, therefore its corneal transparency recorded the highest level, as a response to its nocturnal habitat.

Regarding the arrangement of collagen fibrils, (Chakravarti et al., 1998; Chen and Birk, 2013) proposed the ultimate repulsion forces exist between the dispersed collagen lamellae to prevent corneal opacity and providing transparency. In view of these findings, it seems relative that corneal stroma might be responsible for corneal transparency and formation of sharp retinal. Therefore, any disturbance in the uniform interfibrillar spaces caused loss of transparency. These findings came out in agreement with bovine cornea (Freund et al., 1995; Nishida et al., 1997; Strandberg, 2015; Ling and Bastion, 2018).

It is noticed that moderate density of keratocytes sparsely distributed on the stromal matrix of the mammalian investigated species, was considered as a transparent object except their nuclei. Obviously, there are regular dispersions of keratocytes (small spindle shaped cells), whereas constricted to the anterior portion of the stromal layer with a few densities in case of *R. egyptiacus*. Also, it was uniformly distributed over the whole layer with moderate density in *O. caniculus* and spreading over the stromal of *R. norvigus* with high intensity. Relative distribution is found in rabbits, humans and other different mammals (Lemp et al., 1970; Leonard et al., 2016).

Construction of collagen molecules and proteoglycans is the main task of keratocytes, besides formation of

matrix metalloproteinases (Freund et al., 1995; Meek and Knupp, 2015). Furthermore, acting as transient cells, which activated as macrophage or dendritic cells in case of injury, generating myofibroblasts precursors. Hassell and Birk (2010) observed the same findings in bovine cornea. These justifications supported our results in *R. norvigus* that enables its cornea to accommodate with the highest activity in a hard habitat.

Currently, this study described the keratocytes as light scatter as they are able to change the refractive index for any cytoplasmic alterations; therefore, they increase the corneal opacity and decrease clear transparency. This explains the low density of keratocytes in the nocturnal *R. egyptiacus*. The present findings came in accordance and parallel other land mammals (Jester et al., 1999; Møller-Pedersen, 2004).

Interestingly, only two of the cornea of present mammalian species (*R. egyptiacus* and *O. caniculus*), composed of Bowman's membrane with relatively small thickness, that is considered as a bounded basement membrane, which separates the epithelial layer from the collagen lamellae of the stroma. Besides it appeared as lusterless without any internal structure, however it is identified as a homogenous and excellent transparent elastic Lumina. It was previously reported that the Bowman's membrane is thinner in lower mammals such as rodents and rabbits (Hayashi et al., 2002) and become thicker in higher mammals like human. Similar observation was confirmed in our investigations of *O. caniculus* (lower mammals) and camel and guinea pig (higher mammals) (Merindano, 2002)

Almubrad and Akhtar (2011) confirmed this result in mice, rats, rabbits, cattle, tree shrew and humans. They added that the variation in thickness of the Bowman's membrane might be due to the fixative substance. Furthermore, other histological investigations e.g. Merindano (2002); Hayashi et al. (2002) and Svaldenienė et al. (2003) came in agreement with the same data in higher mammals such as dogs, cattle, human, mouse, rat, guinea pig, rabbit, cat and most carnivores' animals. In contrast to these observations, results of many authors e.g. Ohji et al. (1994); Gibson et al. (1995); Krachmer et al. (1997); El-Dawi, (2005) and Miao et al. (2019) disagree in other mammalian species *Rattus rattus* and *Meriones shawi*.

Its contributions appear in preserving the corneal curvature; rigidity and stiffness. As for accommodation, it is responsible for increasing the refracting power of the cornea, by increasing the corneal curvature to bring the far object closer. Such variation in habitats of the investigated species aroused from opposite light conditions (nocturnal and diurnal habitat) and reflect on the light sensitivity of the eye, morphological structure of cornea and physiological

properties of the retina (Larson, 2004; Jones et al., 2007; Rosencrans, et al., 2018).

Taking into consideration the accommodation range of the flattened cornea, the Bowman's membrane greatly affects in increasing the light sensitivity of nocturnal *R. egyptiacus* more than diurnal *R. norvigus* (which lacks it) and increasing its refracting power, as the vision process estimates the mating process, hiding from enemies and aposematism of the vision. Justification of these findings reported in mouse, rat, guinea pig, rabbit and cattle (Larson, 2004; Rosencrans et al., 2018).

Indeed, there is an optical and physiological correlation between the presence of Bowman's membrane and absorptions of UV, as it acted as an effective filter of UV- radiation with its unique constriction through its arrangement of the collagen fibrils and its molecular composition, to protect the internal structure of the glop from excessive radiation. Furthermore, its absorption coefficient is eight times higher than the coefficient of the stromal layer (Kolozsvári et al., 2002) and (Khan et al., 2019). These explanations illustrated the presence of Bowman's membrane in the cornea of the investigated *O. caniculus* species which inhabited in diurnal conditions. Similar observations reported in higher mammals such as cattle, cat and pig (Hayashi et al., 2002).

The microscopic level referred to the Descemet's membrane observed only in *O. caniculus*, as a specialized basement membrane that separates the substenia propria from cellular endothelium layers with an amorphous appearance in the form of meshwork of homogeneous fibrils. This parallelism was identified in cattle and rabbits (Hayashi et al., 2002).

Obviously, it represented a secretion of the endothelial layer which had integrated function and preserving the corneal stiffness. Similar observations recorded in camel and bovine (Massoudi et al., 2016). As a consequence of the preservations of the morphological features and the integrated function of the endothelium in various physiological conditions, Descemet's membrane acts as a molecular barrier that prevents the infiltration of certain proteins from the aqueous humor into the corneal stroma (Swash and Still, 2005; Chen et al., 2017). The corneal endothelium of three examined species was represented as monolayer of flat squamous epithelial cells. These observations came out in agreement with various vertebrate groups, such as amphibians, reptiles, birds and mammals (Yee et al., 1987; El-Dawi, 2004).

Maintenance of corneal transparency and the integrity of corneal layers depend on the morphological and physiological healthy of the endothelium. A major task of

the endothelium is stromal dehydration and maintaining sodium and potassium ions by an active sodium-potassium pump (Neagu and Petraru, 2015)

Furthermore, it acted as a leaky barrier that transports some ions and macromolecules between nutrient rich aqueous humor and stromal layer against a concentration gradient (Edelhauser, 2006). As mentioned in the previous data, the specialized endothelium has many activated traits, because they have a large number of mitochondria in their cytoplasm, which allows this tissue to be engaged in fluid transportations (Akhtar et al., 2008).

Endothelial population cells that are influenced by age and lesions result in increasing corneal thickness and hydration of the corneal stroma through fluid filtration from the aqueous humor in monkeys, horses, dogs (Tamayo-Arango et al., 2009). Therefore, alterations in the physiological properties of this layer causes corneal opacity and edema.

The high variability in the significance of statistical analysis of corneal thickness in present mammalian species, reflect accommodation with harsh surrounding conditions and various ecological life style (McFarland, 1991). As mentioned in the present study, *O. caniculus* recorded the highest corneal thickness among the studied species.

The present findings reported that the rabbit cornea 480.9  $\mu\text{m}$  which is thicker than frog 80  $\mu\text{m}$  and thinner than of the sheep 600  $\mu\text{m}$ . Therefore, some studies addressed that corneal thickness is directly proportional with the body weight and size (Scott and Bosworth, 1990; Saadi-Brenkia et al., 2018). Besides, Tamayo-Arango et al. (2009) indicated that higher corneal thickness revealed to a large amount of the collagen matrix in the thicker stromal layer that is required for high metabolic activity of the cornea. Another histological explanation was referred that the increase in corneal thickness seems likely to increase the amount of collagen in the stromal layer.

Nevertheless, all corneal layers should be transparent with minimal light scattering property, to achieve the dual function of the cornea. It provides more than of 30% of the refractive power of the eye, and protects the underlying layers of the eye (Meek and Knupp, 2015). Consequently, micro anatomical analysis showed that, the epithelium constitutes relatively about 10% from the corneal thickness. The present study confirmed these findings, in *R. egyptiacus* 10.4% (Reinstein et al., 2008), mouse 11.1% and in cattle 12.5%. These explained that the epithelial layers of the three examined species have non-significant variance ( $P>0.05$ ), whereas Lemp et al. (1970) confirm that result between some mammalian species.



On the other hand, *R. norvigus* had the higher thickness of the two cellular layers (epithelium 13.4% and endothelium 9.3%) as a response to the protection of the rest layers of the eye and preventing the stromal hydration to reduce the corneal opacity and stimulate the corneal transparency. These observations came in accordance and parallel to [Hayashi et al. \(2002\)](#) in most of carnivores species and monkeys.

Regarding the affinity of the mammalian corneal layers to PAS stain, it is observed that the all corneal layers of *R. norvigus* appeared strongly stained. In contrast to other, two investigated species (*R. egyptiacus* and *O. caniculus*), whereas they reported moderate affinity only in the epithelial layer and a strong reaction with PAS stain in the stromal and endothelium layers. Consequently, Bowman's membrane appeared strongly stained in two investigated species (*R. egyptiacus* and *O. caniculus*). On the other hand, Descemet's membrane was completely absent in the three investigated species.

Obviously, the rate of the metabolic activity in the cornea is directly proportional with the corneal glycogen content ([Tamayo-Arango et al., 2009](#)). Therefore, the cellular epithelium and endothelium are highly metabolically activated due to their aerobic glycolysis and oxygen consumption, besides the stromal layer considered as active as the two cellular layers due to its abundance from the polysaccharides ground matrix ([Kinoshita, 1962](#)). This data is consistent with the present observations, and explains the high affinity to PAS reaction in corneal layers of *R. norvigus*. Furthermore, type of habitat and sort of highly activated conditions of this species has an impact on its metabolism and its aerobic glycolysis, besides its molecular organizations of compacted collagen lamellae and its high polysaccharides content ground matrix. Such structure is similar somewhat to that described in diurnal desert rodent *Psammodomys obesus* ([Saadi-Brenkia et al., 2018](#)).

At the molecular level, declining of the corneal opacity and cloudy features result from the homogeneity of all constituents of the cornea ([Meek and Knupp, 2015](#)). This data is ultimately related to the histological composition, life style and the metabolic activity of the cornea, whereas the deficiency in the variability of the epithelial layers of two investigated species (*R. egyptiacus* and *O. caniculus*) emphasize the moderate affinity to PAS stain and stimulation of the homogeneity of the epithelial layer, resulting in corneal transparency. Moreover, the current results confirmed with others in rats, dogs, rabbits and humans ([Meek, 2008](#)).

Accommodation in peculiar habitats of the investigated species frequently stimulated the anatomical modifications of the corneal layers especially in the stromal and

endothelial layers, which enables species' cornea to adapt with their typical ecological surroundings ([Saadi-Brenkia et al., 2018](#)). Nocturnal *R. egyptiacus* possess molecular adaptations by accumulating glycogen in the endothelial layer to supply its high metabolic activity in making the stroma in dehydration state, besides presence of large interfibrillar spaces between its collagen lamellae which in turn improve its corneal transparency. These findings are in accordance with ([Regini et al., 2004](#); [Meek, 2008](#)) in the endothelial layers of cattle and mice cornea.

Despite the stromal layers of diurnal *O. caniculus* packed with glycogen content, which are responsible for supporting the corneal metabolism and its aerobic glycolysis to fit the activation of its large body weight. Currently, high affinity of Bowman's membrane to PAS stain in *O. caniculus* and *R. egyptiacus* referred to molecular organization of elastic lamina (connective tissue packed with glycogen) in the Bowman's membrane. These interpretations and correlations between the content of polysaccharides and the histological compositions referred to highly adaptive range of the investigated species.

The image analysis data revealed that the mammalian cornea of the examined species showed significant variability in their cell density ( $P < 0.000$ ), whereas *R. egyptiacus* possessed the higher statistical variance than other two species. As the current findings indicated that the corneal transparency strongly correlated with the epithelial cell density, besides the corneal thickness, whereas the increasing cell density especially in the epithelial layer, increase the chance in improving the corneal transparency and reducing the corneal cloudy. These findings were consistent with many authors e.g. [Arndt, \(2001\)](#) and [Svaldenienė et al. \(2003\)](#) in dogs and pigs.

Concerning the characteristic features of pleomorphism of the representative examined species that reported a high degree of polygonality in the form of regular polygonal epithelial cell in *R. egyptiacus* and *R. norvigus*, but irregular polygonal cells in *O. caniculus* with distinctly border-like cell breaks. Most probably, the same results reported in cats, monkeys and pigs ([Tamayo-Arango et al., 2009](#))

Pleomorphism traits share in the protection of the corneal epithelium and stabilizing the tear film ([Collin and Collin, 2006](#); [El-Bakry, 2011](#)). Microstructure of the corneal surface demonstrated the correlation between the different ecological surroundings that ranges from the forested habitats (*O. caniculus* and *R. egyptiacus*) to desert arid areas (*R. norvigus*) and various visual conditions (diurnal and nocturnal). Variability in the refractive indices of the corneal epithelial cells when the air surrounded the corneal surfaces (terrestrial species) reflect on the pleomorphism which in turn increase the refractive power of the cornea

through increasing the intensity of the reflected light on the retina (Collin and Collin, 2006). Based on these observations, the current study proposed that the increasing of corneal transparency in cornea of nocturnal *R. egyptiacus* result from its higher pleomorphism and their cell density.

Microtopography of the mammalian cornea distinguished by spreading of microplicae on the corneal surface that sorted into three types light and dark in *R. egyptiacus* and *O. caniculus* and medium and light in *R. norvigus*. Previous attempts tried to resolve presence of microplicae that might be result from various microchanges on the corneal surface, as accommodated response with the different environmental conditions. They developed as precursor of microprojection (microvilli or microridges) on the corneal surface of cat, mouse, sheep (*Ovis aries*) and rabbit (Pfister and science, 1973; Collin and Collin, 2006).

Various explanations that elucidated three types of microplicae could be sorted into light and medium and dark, that might be classified according to their scattering electron on basis of the quantity of microprojections, whereas the light type brings dense microprojection, medium cells covered by moderated microprojections and dark ones have few microprojections (El-Bakry, 2011). These microprojections can be identified based on the maturation of the cell, however at the pre mature phase appear light and also the post metamorphic stage they appeared dark (Pfister and science, 1973; Collin and Collin, 2006). These characteristics to possess high quantity of ascorbic acid in the dark cells to absorb UV- harmful radiations. Alongside, medium cells have a moderate affinity to ascorbic acids, as well as the light cells have a little amount of the acid (Massoudi et al., 2016; Khan et al., 2019).

It is obviously that the cornea of individual investigated species might possess more than one microstructural adaptation trait to preserve their accommodation range. In particular, presence of microplicae is considered a dynamic requirement for healthy habitat preserving stable structure besides, it can change under certain conditions. These observations came in accordance and parallel to that reported in rabbit cornea. The latter case changes from regular erect microplicae in normal conditions to short irregular microplicae, when incubating at various bicarbonate solutions (Doughty, 1990; Khan et al., 2019).

The present study does not recognize any microholes on the corneal surface of the three investigated species. In contrast, previous data identified microholes on the corneal surface of sheep (*Ovis aries*) and Australian kolola (Doughty and Fong, 1992) or represented site of increasing membrane bound vesicles into tear film for stabilizing its structure. It's well known that the main integrated functions of the

microholes are maintaining lubrication of the corneal surface for protection from the high osmolarity medium (aquatic species) and high evaporated habitat (terrestrial species live in high temperature), and reducing fractional rate of the cornea to adapt with quick movement of ocular adnexa (Hayashi et al., 2002). Due to absence of these extreme habitats in the present studied species, microholes did not appear on the epithelial surface. Collin and Collin (2006) referred the absence of the microholes from the mammalian vertebrates to its independent evolution in response to amphibious and burrowing habitats.

Another characteristic feature was noticed, dispersion of the microprojections over the corneal surface of only two investigated species (*R. norvigus* and *O. caniculus*) with high variability in diameters and shapes. On the other hand, corneal surface of *R. egyptiacus* possess blebs (regular spherical microprojections with various diameter). The recent preliminary data of Islam et al. (2019) showed that the design of microprojections is influenced by ocular adnexa like presence or absence of the eyelids and nictating membrane and also by necessity for lubrication and reducing the fraction.

Similar investigations on the cornea of *R. rattus* and *M. shawi* showed that the surface bulged with various diameter blebs, which contributed in the mitotic activity of the epithelium and greatly influenced by direct exposure to extremely surrounding factors of its habitat.

The microprojections and blebs on the investigated corneal surface aid in increasing the surface area for movement of water and metabolic products through cell membrane. In addition, they improve the oxygen consumption and nutrients, which in turn improving the corneal activation of diurnal *R. norvigus* and *O. caniculus*. Also, they can protect the underlying optical layers, and elevated the level of the corneal transparency of the nocturnal *R. egyptiacus*, and as in case of some mammals e.g. cornea of rabbits, cats and pigs (Doughty, 1990, 1991).

Not only the microprojections stabilized the tear film, but also contributed in the refractive power properties which their diversity of patterns stimulating the light capture and increasing the intensity of the light entrance, therefore improving the corneal transparency and preventing corneal opacity.

## CONCLUSIONS AND RECOMMENDATIONS

Various habitats of the investigated species had various significant effects on the histological, histochemical and ultrastructural level of the cornea to accommodate to

different environmental conditions. The acquired data will increase our understanding of the environmental constraints placed on the reptilian cornea and add to our knowledge of the evolutionary development of this tissue. Moreover, to our knowledge, the present study is considered the first to evaluate corneal microstructure in Egyptian's Bats.

## AUTHOR'S CONTRIBUTION

All authors contributed equally.

## ABBREVIATIONS

Epithelium (EP), Stroma (S) and Endothelium (EN), Keratocytes (K), Squamous cell (Sq), Basal columnar cell (Bc), Polyhedral cell (Py), Bowman's membrane (Bm) and Interfibrillar space (IS), Descemet's membrane (DM), Basal cells of epithelial layer (Bc), Regular polygonal epithelial cells (RPC), Irregular polygonal cell (IPC), Light type (L) and Medium type (M) and Dark type (D), Blebs (Bb) and Microprojection (MPJ).

## CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

## REFERENCES

- Abràmoff MD, Magalhães PJ, Ram S (2004). Image processing with ImageJ. *J. Biophoton. Int.*, 11(7): 36-42.
- Akhtar S, Bron A, Salvi S, Hawksworth N, Tuft S, Meek K (2008). Collagen fibrils and proteoglycans in keratoconus—a quantitative ultra-structural analysis. *J. Acta Ophthalmol.*, 86: 764-772. <https://doi.org/10.1111/j.1755-3768.2007.01142.x>
- Akhtar S, Khan AA, Albuhayzah HA, Almubrad TM (2017). Cornea and its adaptation to environment and accommodation function in veiled chameleon (*Chamaeleo calypttratus*): Ultrastructure and 3D transmission electron tomography. *Microsc. Res. Technol.*, 80(6): 578-589. <https://doi.org/10.1002/jemt.22833>
- Almubrad T, Akhtar S (2011). Structure of corneal layers, collagen fibrils, and proteoglycans of tree shrew cornea. *Mol. Vis.*, 17: 2283-2291.
- Arndt C, Reese S, Köstlin R (2001). Preservation of canine and feline corneal scleral tissue in Optisol® GS. *Vet. Ophthalmol.*, 4(3): 175-182. <https://doi.org/10.1046/j.1463-5216.2001.00146.x>
- Bancroft J, Layton C, Bancroft J, Kim Suvarna JS, Layton CDJ (2013). The hematoxylin and eosin, connective and mesenchymal tissues with their stains. pp. 173-212. <https://doi.org/10.1016/B978-0-7020-4226-3.00010-X>
- Brookes NH (2018). Riding the cell jamming boundary: Geometry, topology, and phase of human corneal endothelium. *Exp. Eye Res.*, 172: 171-180. <https://doi.org/10.1016/j.exer.2018.04.007>
- Chakravarti S (2001). The cornea through the eyes of knockout mice. *Exp. Eye Res.*, 73(4): 411-419. <https://doi.org/10.1006/exer.2001.1055>
- Chakravarti S, Magnuson T, Lass JH, Jepsen KJ, LaMantia C, Carroll H (1998). Lumican regulates collagen fibril assembly: Skin fragility and corneal opacity in the absence of lumican. *J. Cell Biol.*, 141(5): 1277-1286. <https://doi.org/10.1083/jcb.141.5.1277>
- Chen J, Li Z, Zhang L, Ou S, Wang Y, He X, Zou D, Jia C, Hu Q, Yang S (2017). Descemet's membrane supports corneal endothelial cell regeneration in rabbits. *Sci. Rep.*, 7(1): 6983. <https://doi.org/10.1038/s41598-017-07557-2>
- Chen S, Birk DE (2013). The regulatory roles of small leucine-rich proteoglycans in extracellular matrix assembly. *J. FEBS J.*, 280(10): 2120-2137. <https://doi.org/10.1111/febs.12136>
- Collin SP, Collin HB (2006). The corneal epithelial surface in the eyes of vertebrates: environmental and evolutionary influences on structure and function. *J. Morphol.*, 267(3): 273-291. <https://doi.org/10.1002/jmor.10400>
- de Oliveira JK, Montiani-Ferreira F, Williams DL (2018). The influence of the tonometer position on canine intraocular pressure measurements using the Tonovet® rebound tonometer. *Open Vet. J.*, 8(1): 68-76. <https://doi.org/10.4314/ovj.v8i1.12>
- DelMonte DW, Kim T (2011). Anatomy and physiology of the cornea. *J. Cataract Refract. Surg.*, 37(3): 588-598. <https://doi.org/10.1016/j.jcrs.2010.12.037>
- Doughty MJ (1990). Analyses of areas and shapes of cells on the corneal surface of the albino rabbit by scanning electron microscopy. *Curr. Eye Res.*, 9(4): 295-306. <https://doi.org/10.3109/02713689008999618>
- Doughty MJ (1990). The ambiguous coefficient of variation: Polymegethism of the corneal endothelium and central corneal thickness. *Int. Contact Lens Clin.*, 17(9-10): 240-248. [https://doi.org/10.1016/0892-8967\(90\)90064-M](https://doi.org/10.1016/0892-8967(90)90064-M)
- Doughty MJ (1991). Scanning electron microscopy study of cell dimensions of rabbit corneal epithelium surface. *Cornea*, 10(2): 149-155. <https://doi.org/10.1097/00003226-199103000-00010>
- Doughty MJ, Fong WK (1992). Topographical differences in cell area at the surface of the corneal epithelium of the pigmented rabbit. *Curr. Eye Res.*, 11(12): 1129-1136. <https://doi.org/10.3109/02713689208999538>
- Edelhauser HF (2006). The balance between corneal transparency and edema the proctor lecture. *Invest. Ophthalmol. Visual Sci.*, 47(5): 1755-1767. <https://doi.org/10.1167/iovs.05-1139>
- El-Bakry AM (2011). Comparative study of the corneal epithelium in some reptiles inhabiting different environments. *J. Acta Zool.*, 92(1): 54-61. <https://doi.org/10.1111/j.1463-6395.2009.00444.x>
- El-Dawi E (2004). Comparative studies on the structural adaptation of the cornea of the amphibious mudskipper fish, *Periophthalmus waltoni* and the maculated toad, *Bufo regularis*. *Egypt. J. Aquat. Biol. Fish.*, 8(2): 207-235.
- El-Dawi ESFA (2005). Comparative studies on the corneal structural adaptation of two rodents inhabiting different environments. *J. Egypt. J. Hospital Med.*, 20: 131-147. <https://doi.org/10.21608/ejhm.2005.18100>
- Freund D, McCally R, Farrell R, Cristol S, L'Hernault N, Edelhauser H (1995). Ultrastructure in anterior and posterior stroma of perfused human and rabbit corneas. Relation to transparency. *Invest. Ophthalmol. Visual Sci.*, 36(8): 1508-1523.
- Gibson MA, Hatzinikolas G, Davis EC, Baker E, Sutherland



- GR, Mecham RP (1995). Bovine latent transforming growth factor beta 1-binding protein 2: molecular cloning, identification of tissue isoforms, and immunolocalization to elastin-associated microfibrils. *Mol. Cell. Biol.*, 15(12): 6932-6942. <https://doi.org/10.1128/MCB.15.12.6932>
- Goldstein JI, Newbury DE, Michael JR, Ritchie NW, Scott JHJ, Joy DC (2017). Scanning electron microscopy and X-ray microanalysis, Springer. [https://doi.org/10.1007/978-1-4939-6676-9\\_27](https://doi.org/10.1007/978-1-4939-6676-9_27)
  - Hassell JR, Birk DE (2010). The molecular basis of corneal transparency. *J. Eye Res.*, 91(3): 326-335. <https://doi.org/10.1016/j.exer.2010.06.021>
  - Hayashi S, Osawa T, Tohyama K (2002). Comparative observations on corneas, with special reference to Bowman's layer and Descemet's membrane in mammals and amphibians. *J. Morphol.*, 254(3): 247-258. <https://doi.org/10.1002/jmor.10030>
  - Hutcheon AE, Zieske JD, Guo X (2019). 3D in vitro model for human corneal endothelial cell maturation. *Exp. Eye Res.*, 184: 183-191. <https://doi.org/10.1016/j.exer.2019.04.003>
  - Iannaccone S, Zhou Y, Walterhouse D, Taborn G, Landini G, Iannaccone P (2012). Three dimensional visualization and fractal analysis of mosaic patches in rat chimeras: cell assortment in liver, adrenal cortex and cornea. *PLoS One*, 7(2): e31609. <https://doi.org/10.1371/journal.pone.0031609>
  - Islam MM, Sharifi R, Gonzalez-Andrades M (2019). Corneal tissue engineering. Corneal regeneration, Springer: 23-37. [https://doi.org/10.1007/978-3-030-01304-2\\_3](https://doi.org/10.1007/978-3-030-01304-2_3)
  - Jeffrey CE, Read ND (1991). Ambient-and low-temperature scanning electron microscopy. *J. Electron. Microsc. Plant Cells*, 313: 413. <https://doi.org/10.1016/B978-0-12-318880-9.50013-2>
  - Jester JV, Moller-Pedersen T, Huang J, Sax CM, Kays WT, Cavanagh HD, Petroll WM, Piatigorsky J (1999). The cellular basis of corneal transparency: Evidence for 'corneal crystallins'. *J. Cell Sci.*, 112(5): 613-622. <https://doi.org/10.1242/jcs.112.5.613>
  - Jones MP, Pierce Jr KE, Ward D (2007). Avian vision: A review of form and function with special consideration to birds of prey. *J. Exot. Pet. Med.*, 16(2): 69-87. <https://doi.org/10.1053/j.jepm.2007.03.012>
  - Khan M, Sarwar MS, Jahangir S (2019). Comparison of central and peripheral corneal thickness in different degree of myopic patient. *J. Ophthalmol. Pak.*, 8(03): 24-27.
  - Kinoshita JH (1962). Some aspects of the carbohydrate metabolism of the cornea. *J. Investig. Ophthalmol. Visual Sci.*, 1(2): 178-186.
  - Kolozsvári L, Nógrádi A, Hopp B, Bor Z (2002). UV absorbance of the human cornea in the 240-to 400-nm range. *J. Invest. Ophthalmol. Visual Sci.*, 43(7): 2165-2168.
  - Krachmer JH, Mannis MJ, Holland EJ (1997). Cornea: Fundamentals of cornea and external disease, Mosby.
  - Land MF, Nilsson DE (2012). Animal eyes, Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780199581139.001.0001>
  - Larson KA (2004). Advertisement call complexity in northern leopard frogs, *Rana pipiens*. *J. Copeia*, 2004(3): 676-682. <https://doi.org/10.1643/CH-03-247R2>
  - Lemp MA, Holly FJ, Iwata S, Dohlman CH (1970). The precorneal tear film: I. Factors in spreading and maintaining a continuous tear film over the corneal surface. *J. Arch. Ophthalmol.*, 83(1): 89-94. <https://doi.org/10.1001/archophpt.1970.00990030091017>
  - Leonard BC, Yañez-Soto B, Raghunathan VK, Abbott NL, Murphy CJ (2016). Species variation and spatial differences in mucin expression from corneal epithelial cells. *J. Exp. Eye Res.*, 152: 43-48. <https://doi.org/10.1016/j.exer.2016.09.001>
  - Ling K, Bastion MLC (2018). Use of commercially available sodium hyaluronate 0.18% eye drops for corneal epithelial healing in diabetic patients. *J. Int. Ophthalmol.*, pp. 1-9. <https://doi.org/10.1007/s10792-018-1057-1>
  - Lombardi L, Fernández N, Moreno S, Villafuerte R (2003). Habitat-related differences in rabbit (*Oryctolagus cuniculus*) abundance, distribution, and activity. *J. Mammal.*, 84(1): 26-36. [https://doi.org/10.1644/1545-1542\(2003\)084<0026:HRDIRO>2.0.CO;2](https://doi.org/10.1644/1545-1542(2003)084<0026:HRDIRO>2.0.CO;2)
  - Malmström T, Kröger RH (2006). Pupil shapes and lens optics in the eyes of terrestrial vertebrates. *J. Exp. Biol.*, 209(1): 18-25. <https://doi.org/10.1242/jeb.01959>
  - Massoudi D, Malecaze F, Galiacy SD (2016). Collagens and proteoglycans of the cornea: Importance in transparency and visual disorders. *J. Cell Tissue Res.*, 363(2): 337-349. <https://doi.org/10.1007/s00441-015-2233-5>
  - McFarland WN (1991). The visual world of coral reef fishes. The ecology of fishes on coral reefs, Elsevier, pp. 16-38. <https://doi.org/10.1016/B978-0-08-092551-6.50007-6>
  - McManus J (1946). Histological demonstration of mucin after periodic acid. *J. Nat.*, 158(4006): 202. <https://doi.org/10.1038/158202a0>
  - Meek K (2008). The cornea and sclera. Collagen, Springer: 359-396. [https://doi.org/10.1007/978-0-387-73906-9\\_13](https://doi.org/10.1007/978-0-387-73906-9_13)
  - Meek KM, Knupp C (2015). Corneal structure and transparency. *Prog. Retin. Eye Res.*, 49: 1-16. <https://doi.org/10.1016/j.str.2014.11.011>
  - Merindano M (2002). A comparative study of Bowman's layer in some mammals: Relationships with other constituent corneal structures. *Eur. J. Anat.*, 6: 8.
  - Miao S, Lin Q, Liu Y, Song Y-W, Zhang Y-N, Pan Z-Q (2019). Clinicopathologic features and treatment characteristics of congenital corneal opacity infants and children aged 3 years or less: A retrospective single institution analysis. *Med. Principles Pract.*, 29: 18-24. <https://doi.org/10.1159/000501763>
  - Mickleburgh SP, Hutson AM, Racey PA (1992). Old world fruit bats. An action plan for their conservation. Gland, Switzerland: IUCN 263. pp. 211-232. <https://doi.org/10.2305/IUCN.CH.1992.SSC-AP.6.en>
  - Møller-Pedersen T (2004). Keratocyte reflectivity and corneal haze. *J. Exp. Eye Res.*, 78(3): 553-560. [https://doi.org/10.1016/S0014-4835\(03\)00208-2](https://doi.org/10.1016/S0014-4835(03)00208-2)
  - Morris JK (1965). A formaldehyde glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.*, 27: 1A-149A.
  - Murata K, Hirata A, Ohta K, Enaida H, Nakamura KI (2019). Morphometric analysis in mouse scleral fibroblasts using focused ion beam/scanning electron microscopy. *Sci. Rep.*, 9(1): 6329. <https://doi.org/10.1038/s41598-019-42758-x>
  - Nagayasu A, Hirayanagi T, Tanaka Y, Tangkawattana P, Ueda H, Takehana K (2009). Site-Dependent differences in collagen lamellae in the corneal substantia propria of Beagle dogs. *J. Vet. Med. Sci.* 71(9): 1229-1231. <https://doi.org/10.1292/jvms.71.1229>
  - Neagu A, Petraru O (2015). Aquatic vs terrestrial eye design. A functional ecomorphological approach. *J. Analele S. tiințifice*

- ale Universitații,, Alexandru Ioan Cuza” din Ias, i, s. Biol. Anim., 61: 101-115.
- Nejad TM, Iannaccone S, Rutherford W, Iannaccone PM, Foster C (2015). Mechanics and spiral formation in the rat cornea. *Biomech. Model. Mechanobiol.*, 14(1): 107-122. <https://doi.org/10.1007/s10237-014-0592-6>
  - Nishida K, Honma Y, Dota A, Kawasaki S, Adachi W, Nakamura T, Quantock AJ, Hosotani H, Yamamoto S, Okada M (1997). Isolation and chromosomal localization of a cornea-specific human keratin 12 gene and detection of four mutations in Meesmann corneal epithelial dystrophy. *J. Am. J. Hum. Genet.*, 61(6): 1268-1275. <https://doi.org/10.1086/301650>
  - Ohji M, Sundar RN, Hassell JR, Thoft RA (1994). Basement membrane synthesis by human corneal epithelial cells *in vitro*. *J. Invest. Ophthalmol. Visual Sci.*, 35(2): 479-485.
  - Parekh M, Peh G, Mehta JS, Ahmad S, Ponzin D, Ferrari S (2019). Effects of corneal preservation conditions on human corneal endothelial cell culture. *Exp. Eye Res.*, 179: 93-101. <https://doi.org/10.1016/j.exer.2018.11.007>
  - Passo RM, Hoskins ZB, Tran KD, Patzer C, Edmunds B, Morrison JC, Parikh M, Takusagawa HL, Tehrani SJO, Therapy (2019). Electron beam irradiated corneal versus gamma-irradiated scleral patch graft erosion rates in glaucoma drainage device surgery. pp. 1-6. <https://doi.org/10.1007/s40123-019-0190-x>
  - Pfister RR, Science V (1973). The normal surface of corneal epithelium: a scanning electron microscopic study. 12(9): 654-668.
  - Pourlis AF (2013). Scanning electron microscopic studies of the pecten oculi in the quail (*Coturnix coturnix japonica*). *J. Anat. Res. Int.*, 2013:1-6. <https://doi.org/10.1155/2013/650601>
  - Regini JW, Elliott G, Hodson SA (2004). The ordering of corneal collagen fibrils with increasing ionic strength. *J. Mol. Biol.*, 336(1): 179-186. <https://doi.org/10.1016/j.jmb.2003.12.001>
  - Reinstein DZ, Gobbe M, Archer TJ, Silverman RH, Coleman DJ (2008). Epithelial thickness in the normal cornea: three-dimensional display with Artemis very high-frequency digital ultrasound. *J. Refract. Surg.*, 24(6): 571-581. <https://doi.org/10.3928/1081597X-20080601-05>
  - Rosencrans RF, Leslie CE, Perkins KA, Walkowski W, Gordon WC, Richards-Zawacki CL, Bazan NG, Farris HE (2018). Quantifying the relationship between optical anatomy and retinal physiological sensitivity: A comparative approach. *J. Comp. Neurol.*, 526(18): 3045-3057. <https://doi.org/10.1002/cne.24531>
  - Saadi-Brenkia O, Hanniche N, Lounis S (2018). Microscopic anatomy of ocular globe in diurnal desert rodent *Psammomys obesus* (Cretzschmar, 1828). *J. Basic Appl. Zool.*, 79(1): 43. <https://doi.org/10.1186/s41936-018-0056-0>
  - Saleh MA (1997). Amphibians and reptiles of Egypt, Arab Republic of Egypt, Cabinet [sic] of Ministers, Ministry of State for Environmental Affairs, Egyptian Environmental Affairs Agency.
  - Scott J, Bosworth T (1990). A comparative biochemical and ultrastructural study of proteoglycan-collagen interactions in corneal stroma. Functional and metabolic implications. *J. Biochem. J.*, 270(2): 491-497. <https://doi.org/10.1042/bj2700491>
  - Sheppard J, Hayes S, Boote C, Votruba M, Meek KM (2010). Changes in corneal collagen architecture during mouse postnatal development. *Invest. Ophthalmol. Visual Sci.*, 51(6): 2936-2942. <https://doi.org/10.1167/iov.09-4612>
  - Strandberg H (2015). The world as resistance. *Self-Knowledge and Self-Deception*, Springer, pp. 135-146. [https://doi.org/10.1057/9781137538222\\_10](https://doi.org/10.1057/9781137538222_10)
  - Suvarna S, Layton C (2013). Bancroft's theory and practice of histological techniques. Churchill livingstone. J Elsevier. Aughey E, Frye FL. Comparative veterinary histology. Manson Publ., 21(2010): 173-186. <https://doi.org/10.1016/B978-0-7020-4226-3.00005-6>
  - Svaldenienė E, Paunksnienė M, Babrauskienė V (2003). Appliance of digital ultrasonic technique in canine cornea investigations. *Ultragarsas "Ultrasound"* 46(1): 41-45.
  - Swash A, Still R (2005). Birds, mammals, and reptiles of the Galápagos Islands: An identification guide, Yale University Press.
  - Tamayo-Arango LJ, Baraldi-Artoni SM, Laus JL, Vicenti FAM, Pigatto JA, Abib FC (2009). Ultrastructural morphology and morphometry of the normal corneal endothelium of adult crossbred pig. *J. Ciência Rural*, 39(1): 117-122. <https://doi.org/10.1590/S0103-84782009000100018>
  - Yee RW, Edelhauser HF, Stern ME (1987). Specular microscopy of vertebrate corneal endothelium: A comparative study. *J. Exp. Eye Res.*, 44(5): 703-714. [https://doi.org/10.1016/S0014-4835\(87\)80141-0](https://doi.org/10.1016/S0014-4835(87)80141-0)