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Analysis of the kinetics of the eyelids of little owl *Athene noctua*

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Abstract

This study gives a comprehensive description of eyelids movement in little owl and discusses the impact of some surrounding conditions in their kinetic performance. The present study used the video's recording technique to record the kinetic activity of eyelids, besides the anatomical and histological studies of the eyelid's structure. The fundamental eyelid movements can be uniquely and reliably characterized by their anatomical relationship that was confirmed via video recording for their kinetic activity. The levator palpebrae muscle is considered a main generating motor for the upper eyelid; in the little owl, this muscle splits into multiple directions and is distinguished from the levator palpebrae superioris (Lps) and the levator anguli oculi (Lao) muscle. That anatomical pattern of insertion increases the movement of the upper lid. On the other side, the contraction of depressor palpebrae inferioris (Dpi) muscle and the active upward forces of levator palpebrae muscle help in increasing the opening of the eye's fissure. However, the closure process is produced from the passive downward forces and relaxation of the levator palpebrae superioris (Lps), levator anguli oculi (Lao), and depressor palpebrae inferioris muscle, as well as the contraction of retractor anguli oculi lateralis (Raol) and medialis (Raom) muscle. The present results also recorded that nictitating membrane's (Nm) movement is reversely proportionate to the level of kinetic of other eyelids. The mobility of Nm in little owl occurs under the effect of artificial external stress. These anatomical data and sequence video recordings have confirmed that the upper eyelid moves more compared to other eyelids. The authors also suggest that the mobility of eyelids may get stimulated through external pressure force of some surrounding structure like the periorbital sheet. Also, the histological study exhibited that the structure of two eyelids is very similar in the little owl and the variability is showing in the number of cell layers that forms their epithelium of skin and palpebral surfaces, the distribution of pigment granules, and degree of keratinization on their surface. That variability in the histological characters of eyelids may counteract the abrasive forces occurring during the opening and closing processes.

KEYWORDS

anatomy, eyelids, kinetic, little owl, periorbital sheet

1 | INTRODUCTION

Owls have different activity patterns, from nocturnal to crepuscular or cathemeral, or to diurnal. The little owl is a predator bird, hunting mainly at night but also active during daylight (Zerunian et al., 1982). In 2008, Hall classified the little owl as phototropic birds (diurnal species). Obviously, the activity pattern of the little owl is still under debate and needs more study to confirm it. The difference in activity pattern is reflected in the structure of visual system of owls (Gutiérrez-Ibáñez et al., 2012). Several previous studies have mentioned the structure of the visual system of some other owl species, but we noticed that little owl was not given much of such attention.

The protective apparatus of eye (ocular adnexa) includes lids, glands, and muscles. This apparatus revealed significant differences in birds and reptiles, and in mammals that reflect a wide range of the activity patterns (Klećkowska-Nawrot et al., 2016, 2017; Schramm et al., 1994). The three eyelids are a common feature in birds in reptiles, and in some mammals (Jochems & Phillips, 2015). Many previous studies have concluded that the upper eyelid in different bird species is thick and short, while the lower eyelid is thinner, longer, and moveable (Hall et al., 2009; Jochems & Phillips, 2015). They also recorded that the third eyelid is an elastic membrane and moves rapidly. The main purpose of this apparatus is to cleanse and lubricate the front of the cornea, as well as, to act as an immune protection for the eye (Klećkowska-Nawrot et al., 2017).

Klećkowska-Nawrot et al. (2017) recorded wide variations in the microstructure of eyelids in many wild bird species. These recent studies confirmed that the eyelids of birds differ in their structure among different avian species. For this reason, the present study attempts to give more details about the structure of eyelids of some birds which had been neglected in this field of study, like the little owl *Athene noctua*, and discuss the influence of some conditions in kinetic performance of these eyelids.

2 | MATERIAL AND METHODS

Ten adult specimens of little owl, *Athene noctua*, were brought to the comparative anatomy of vertebrate laboratory, Zoology Department, Faculty of Science, Assiut University, in a condition of good health, and cared for according to the guidelines of the Research Ethics Committee, Assiut University (www.enrec.org). The specimens of little owl were placed in a wide cage and a camera was installed in one of the corners of the cage for a period of 24 h to follow the natural movement of the eyelids without any external influence. The next day, the specimens were caught and the eyelids (upper and lower) were fixed using the fingers of the hand and the resulting movement was recorded. Then these ten specimens were divided for the following procedures.

2.1 | Anatomical study

The heads of five specimens were fixed in 10% formalin for two weeks and then stored in 2% phenoxy-ethanol for long-term preservation. Photographs were taken using a Toup camp XCAM full HD camera. The present study followed Nomina Anatomica Veterinaria (2017) for anatomical terminology of the eyelid's musculature system and cranial skeleton.

2.2 | Histological study

Three specimens of eyelids were fixed in 10% neutral formalin for three days, then put in 70% ethyl alcohol for two days, after which they were dehydrated through a series of ethyl alcohols, cleared in methyl benzoate for three days, embedded in paraffin wax, sectioned serially (7 μm) and then sections were stained with Haematoxylin and Eosin, Masson's trichromic stain, and periodic acid-Schiff (PAS; Drury & Wallington, 1980). For transmission electron microscopic investigation (TEM), parts from the eyelids were cut into small pieces (1 mm each) and were fixed in a cacodylate-buffered solution of 5% glutraldehyde for 2 h, then washed several times in the same buffer for 1 hr at pH = 7.2, and then, the specimens were post fixed in a cacodylate buffer 1% osmium tetraoxide for 2 h at 4°C. Specimens were washed several times in the second step, followed by dehydration in graded series of alcohol. The specimens were embedded in epoxy resin; they were treated for semi-thin sectioning at 1 mm thickness and stained with toludine blue for light microscopic examination. Photographs were taken with an Olympus camera model DP74 connected with Olympus microscope model BX43.

2.3 | Scanning electron microscopy study

The eyes of two specimens were cut into small pieces and directly fixed in 5% glutaraldehyde in a cacodylate buffer for 48 h at 4°C and washed in three changes of 0.1% cacodylate buffer, and then, the specimens were post-fixed in a cacodylate-buffered solution of 1% osmium tetroxide for 2 h at 37°C. The specimens were washed in the same buffer three times, dehydrated through ascending series of ethyle alcohol, and then infiltrated with amyl acetate for two days. The drying of the specimens was accomplished by the critical point drying using liquid carbon dioxide, mounted and sputter-coated with gold. The specimens were examined on a Jeol scanning electron microscope (J S M-5400I V) at 15 kv.

3 | RESULTS

The eyelids of the little owl *Athene noctua*, were distinguished into upper, lower, and third eyelids.

3.1 | The anatomical investigation of the upper and lower eyelids of little owl

The little owl, *Athene noctua*, has movable eyelids (upper eyelid and lower eyelid). The upper eyelid is shorter, thicker, and highly movable, more than the lower one (Figures 1 and 2). The palpebral margin of each eyelid is deeply pigmented and segmented into a number of folds. The anatomical investigation of the eyelids of the little owl reveals that the anterior edge of palpebral margin of upper lid is raised into voluminous ridges which give sausage-like segments. These segments decrease in size posteriorly and convert into shallow irregular ridges (Figure 1b). The palpebral margin of the lower eyelid bears relatively larger anterior segment than that of its posterior edge (Figure 1a). Moreover, the palpebral margin of each eyelid carries two rows of modified feathers as eyelashes (Figure 1a,b).

3.2 | Histological and scanning electron microscopic investigations of the upper eyelid

Histological investigation of the upper eyelid of little owl, *Athene noctua*, showed that the skin surface of the upper lid is composed of keratinized stratified squamous epithelium with three or four nucleated cell layers (Figure 3a) which multiply into ten layers to form the palpebral margin of the upper eyelid of little owl lacking keratinization (Figure 3b). Numerous pigmented granules fill the cytoplasm of these cell layers of skin surface which increase in palpebral margin of the upper eyelid (Figure 3b).This skin epithelium is inclined inwards to form the conjunctival surface of the upper eyelid which changes from stratified squamous type to stratified cuboidal (Figure 3c). The epithelium of conjunctival surface contains numerous goblet cells, which exhibits purple colour with PAS reaction and slightly bluish colour with toluidine blue stain (Figure 3c,d).

The stroma of the upper eyelid contains a scattered network of collagen fibres with less abundant elastic ones,

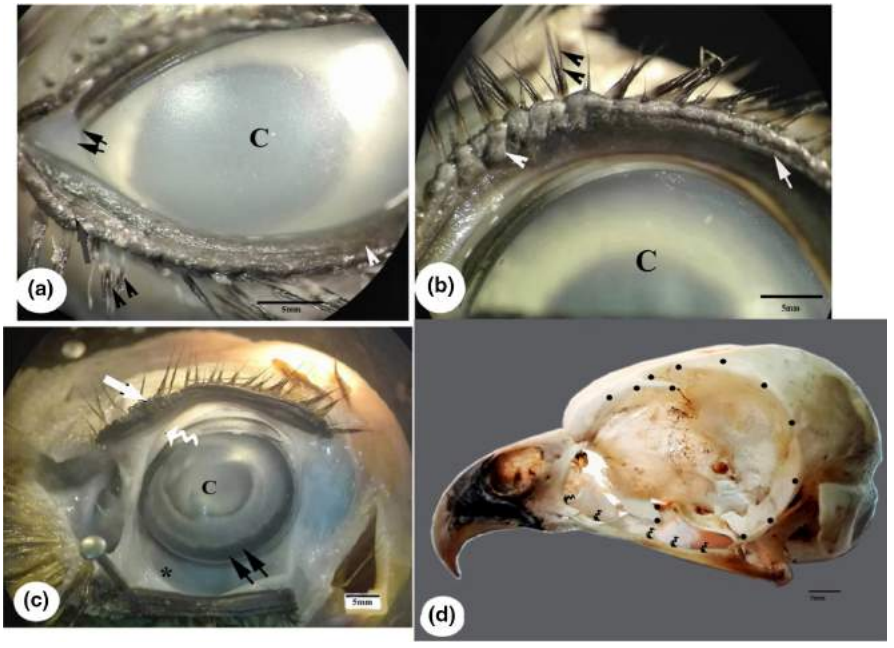

FIGURE 1 Photomacrograph of the eye of little owl, *Athene noctua*, showing: (a) the palpebral margin of the lower eyelid with large anterior segment (arrow), small posterior segment (arrow head), two rows of feather eyelashes (double arrows head), and the fixed point of the nictitating membrane (double arrows). (b) the palpebral margin of the upper eyelid with sausage-like segments (arrow head), shallow irregular ridges (arrow), feather eyelashes (double arrows head), and the cornea (c). (c) the folds of nictitating membrane (zigzag arrow), upper eyelid (white arrow), and lower eyelid (star), cornea (C), and iridocorneal junction (double arrows). (d) the attachment site of the periorbital sheet on the skull (dark spots) and indirectly attaches with some movement of bony elements of the skull (zigzag arrow).

FIGURE 2 Photo of the sequence video record of movement of eyelids of little owl, *Athene noctua*, in natural condition.

a sparse number of melanocytes and blood vessels. Also, clusters of adipocytes fill the stroma of the upper eyelid, but few within its margin (Figure 3e,f).

Semi-thin sections of the mucosa of skin surface of the upper lid reveals the following main layers: stratum basal in which the basal cell layer contains rounded and oval nuclei; the stratum intermedium that forms two layers of polyhedral cells outer to the basal layer; the stratum transitivum that lies outer to the stratum intermedium and is formed of flattened cells with oval nuclei; and the stratum corneum is formed of wavy keratin which is lacking in nuclei (Figure 3a).

Scanning electron microscopy investigation reveals the presence of heavy detached keratin on the skin surface of upper lid (Figure 5a). In addition, the appearance of goblet cells is scattered on the conjunctival surface of the upper eyelid with their secretion spreading on its surface. Also, the intercellular borders are clearly observed (Figure 5b).

3.3 | Histological and scanning electron microscopic investigations of the lower eyelid

The structure of skin and conjunctival surfaces (Cs) of the lower eyelid is similar to that of the upper eyelid. The skin surface of lower eyelid of little owl is composed of stratified squamous epithelium which consists of one or two nucleated cell layers and is covered by thin keratin (Figure 4a–c). These epithelial layers increase gradually inwards to the conjunctival surface to become ten non-keratinized nucleated cell layers, and also, their cytoplasm contains highly dense dark pigment granules, especially at the palpebral margin (Figure 4d).

The epithelial layer that forms the conjunctival surface near the palpebral margin of the lower eyelid is covered by a thick keratinized layer. This thick keratinized layer returns only to disappear along the whole length of the conjunctival surface (Figure 4b,e,f).

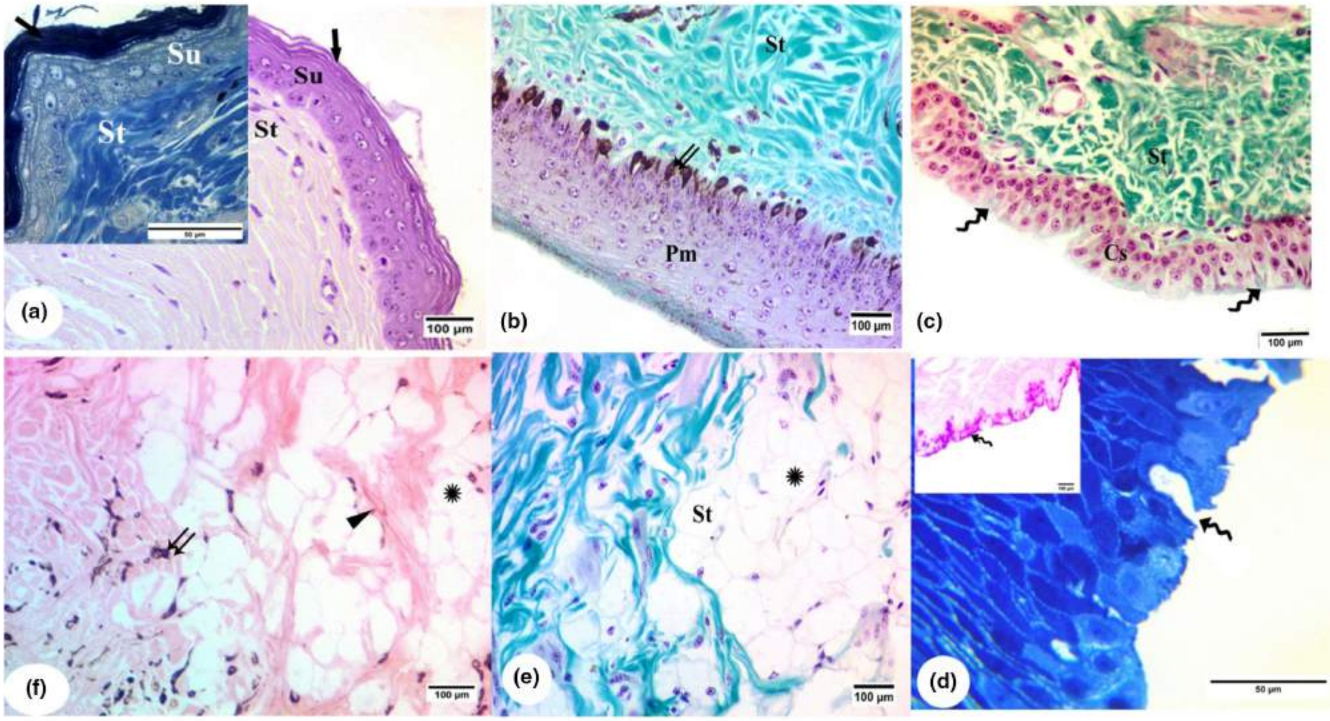

FIGURE 3 Photomicrograph of a transverse section of upper eyelid of little owl, *Athene noctua*, showing: (a) the main layers of skin surface (Su) is composed of stratified squamous epithelium covers with wavy keratin (arrow); upper left, show the parallel distribution of fibres beneath the surface (a by H&E; upper left by toluidine blue, scale bar, 100, 50 µm). (b) the palpebral margin (Pm), numerous pigmented granules (double arrows), the collagen fibres within stroma (St) (Masson's trichromic, scale bar, 100 µm). (c and d) the conjunctival surface (Cs) with goblet gland (zigzag arrow) (c by Masson's trichromic and d by toluidine blue, upper left by PAS, scale bar, 100, 50 µm). (e and f) scattered network of fibres within stroma (St); collagen fibres (green colour), elastic fibres (arrowhead) and clusters of adipocytes (star) (e by Masson's trichromic, f by Orcien, scale bar, 100 µm).

The conjunctival surface of the lower eyelid of little owl is covered by stratified cuboidal epithelium with few goblet cells (Figure 4e). The plate of dense collagen fibres (inferior tarsal plate) is present beneath the conjunctival epithelium. That tarsal plate is invisible in the upper eyelid (Figure 4f).

The stroma of the lower eyelid is filled with parallel collagen fibres, which just underlies the skin surface of the lower eyelid and then becomes irregularly distributed at the palpebral margin and beneath the conjunctival surface. It has been observed that numerous melanocytes, blood vessels, and clusters of adipocytes are scattered within this stroma, but the later one is less than that of the upper eyelid (Figure 4).

Scanning electron microscopy investigation reveals the presence of heavy detached keratin on the skin surface of the lower lid (Figure 5c,d), which disappears along the whole length of the conjunctival surface (Figure 5e,f). Moreover, the conjunctival surface of the lower eyelid of little owl reveals an appearance of goblet cells with their secretion spread on its surface and the intercellular borders are clearly observed (Figure 5e,f).

3.4 | The anatomical investigations of the third eyelid (nictitating membrane)

The nictitating membrane of little owl is a well-developed semi-transparent and mobile organ which is formed as the re-duplication of conjunctiva (Figure 1c). That membrane possesses two surfaces: the external surface which faces the conjunctiva surface of the upper eyelid (Palpebral surface) and merges superiorly with the fornix conjunctiva palpebrae (Figure 6a) and the internal one faces the cornea (Bulbar surface) which merges with the fornix conjunctiva bulbi (Figure 6b). The free margin of this membrane is a thick and dark edge which is extended obliquely to the anterior canthus, which is almost visible during rest of action beneath the palpebral margin of the upper eyelid (Figure 1a–c).

During observation of the little owl in the laboratory, the movement of the nictitating membrane of little owl showed that the membrane slips rapidly and obliquely across the front of the eye from a fixed point at the anterior canthus and the fornix conjunctiva palpebrae towards

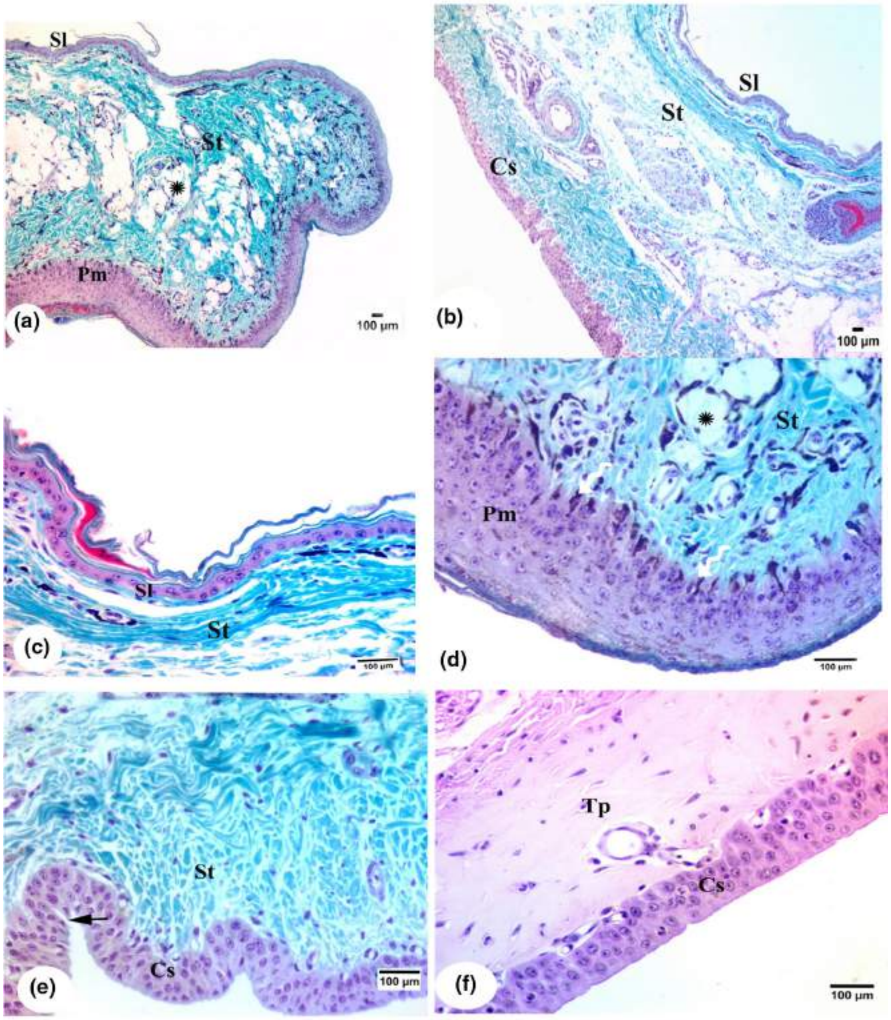

FIGURE 4 Photomicrograph of a transverse section of the lower eyelid of little owl, *Athene noctua*, showing: (a) the change in thickness of epithelium and keratinization along the lower eyelid, stroma (St) contains highly dense of dark pigment granules (zigzag arrow) especially at the palpebral margin (Pm) and adipocytes (star). (b) skin surface or lower eyelid (SI), stroma (St) with parallel collagen fibres. (c) the palpebral margin (Pm) highly dense of dark pigment granules (zigzag arrow) and adipocytes (star). (d) the conjunctival surface (Cs) with evidence goblet gland (arrow). (f) inferior tarsal plate (Tp). (a, b, c, d, e by Masson's trichromic, f by H & E, scale bar, 100 µm).

the ventro-temporal direction. This movement occurs when the movement of eyelids is fixed and observed from the anterior angle of membrane slips beneath the lower eyelid (Figure 7).

3.5 | Histological and scanning electron microscopic investigations of the nictitating membrane

Histological investigation of the nictitating membrane of little owl reveals that both surfaces of the nictitating membrane are covered by folded stratified epithelium (Figure 6a,b). The free margin and palpebral surface of the nictitating membrane are covered by non-keratinized squamous epithelium (Figure 6c,e) while its bulbi surface is covered by stratified columnar epithelium with dome shape (Figure 6f). The epithelium of bulbi surface just near the free margin carries a long cytoplasmic extension like the feather duster (Figure 6d,f). Moreover, numerous pigmented granules are interspersed only between the epithelial cells of the free margin (Figure 6c).

Semi-thin sections of the nictitating membrane of little owl reveal that the epithelium of the palpebral surface and the intermediate cells are flattening superficially to produce squamous cell layers with oval nuclei and pale cytoplasm (Figure 6d). On bulbi surface, the apical membrane of these intermediate cells is bulging to form dome-shaped cells with apical cytoplasm extension which are well observed by SEM (Figure 8c). That cytoplasm extension decreases in density towards the fornix conjunctiva bulbi (Figure 8d). Furthermore, the epithelium of fornix conjunctiva bulbi intersperses with numerous unicellular mucous glands which exhibits positive reaction with PAS (Figure 6g,h).

Scanning electron microscopy investigation of the nictitating membrane of little owl observed that the surface of this membrane becomes more wrinkled medially than its anterior and posterior fixed point (at anterior and posterior canthus; Figure 8a). The bulbi surface of this membrane has dome-shaped cells interspersed with scattered pores (Figure 8c), and then this surface becomes more flattened towards fornix conjunctiva (Figure 8d).

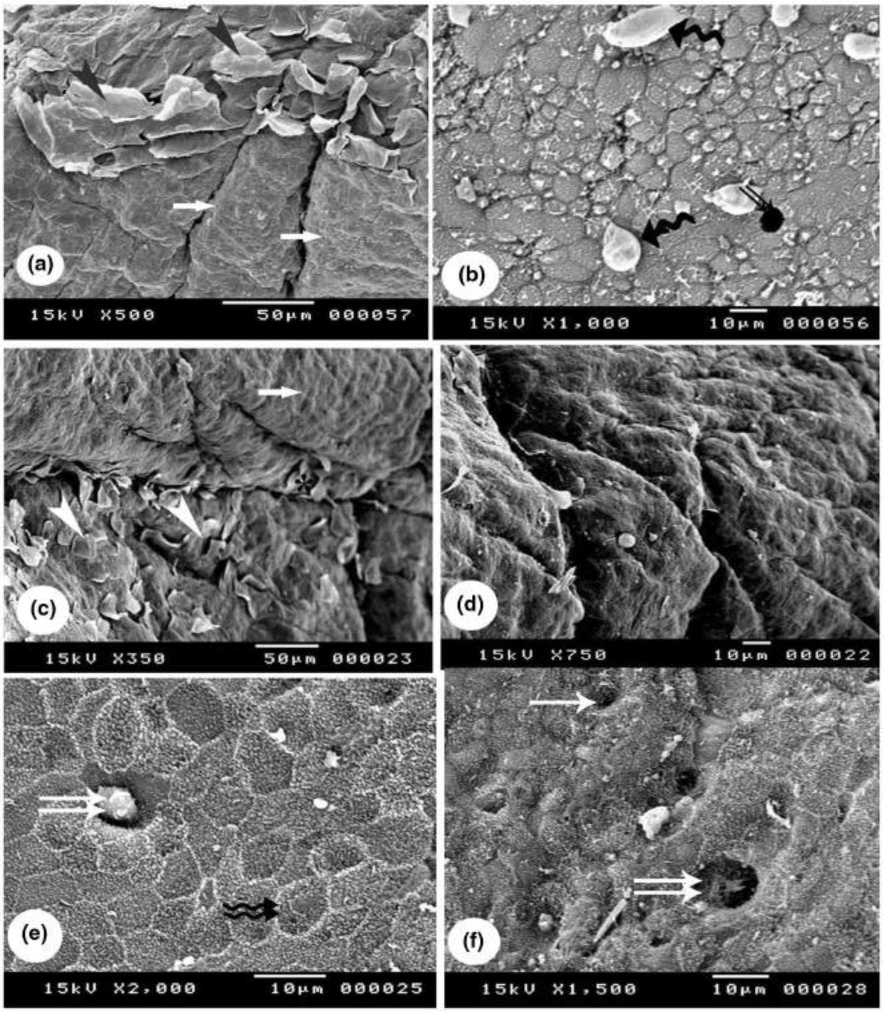

FIGURE 5 Scanning electron micrograph eyelids of the little owl, *Athene noctua*, showing: (a) the detached keratin in the skin surface of upper eyelid (arrowhead) which convert into smooth surface of the palpebral margin (arrow). (b) the conjunctival surface of the upper eyelid, pore of goblet cells (double arrows) and its secretion on the surface (zigzag arrow). (c) the skin surface of lower eyelid with deciduous keratin (arrowhead) which disappears towards the conjunctival surface of the lower eyelid (arrow). (d) the smooth palpebral margin of lower eyelid. (e and f) the cell border of the conjunctival surface of lower eyelid (double zigzag arrows), and the presence of goblet cell (double arrows).

3.6 | The anatomical investigation of eyelids muscle

These muscles control the movement of the eyelids and nictitating membrane that includes muscle levator palpebrae superioris (LPS), levator anguli oculi (LAO), retractor anguli oculi lateralis (RAOL), retractor anguli oculi medialis (RAOM), depressor palpebrae inferioris (DPI), quadratus (Qm), and pyramidalis (Pm) (Figure 9 and Table 1).

All these muscles are covered completely by the periorbital sheet which is a white strong sheet, appearing as parallel bundles of collagen fibres beneath the skin surface of eyelids (Figures 3a and 4c).

3.7 | Levator palpebrae superioris muscle

Levator palpebrae superioris is sheet-like with parallel muscle fibres that run dorsal to the rectus dorsalis muscle. The Levator palpebrae superioris extends dorso-laterally from its origin on the interorbital septum posterior to the origin site of the oblique dorsalis muscle. The muscle fibres of this muscle fan out laterally to be inserted into the connective tissue of the upper eyelid. During its divergence, the muscle fibres interfere with the antero-dorsal fibres of the muscle levator anguli oculi and underpass the glandula lacrimalis hides completely by the periorbital sheet which is located on the interorbital septum just then these muscles and interfere with and during its dorsolateral diverge the muscles of the levator palpebrae superioris.

3.8 | Levator anguli oculi muscle

Levator anguli oculi is a sheet like, parallel fibred muscle which is located posterior-lateral to the muscle levator palpebrae superioris and anterior to the muscle levator anguli oculi lateralis. The muscle levator anguli oculi originates from the posterio-lateral edge of the parietal bone (anterior temporal crest) via thin aponeurosis and fleshy muscle fibres, just anterior to the origin site of the muscle retractor anguli lateralis. Then, the muscle levator anguli oculi runs obliquely antero-lateral to be inserted on the connective tissue of the upper eyelid and nictitating membrane and emerges with the muscle fibres of the Levator Palpebrae Superioris.

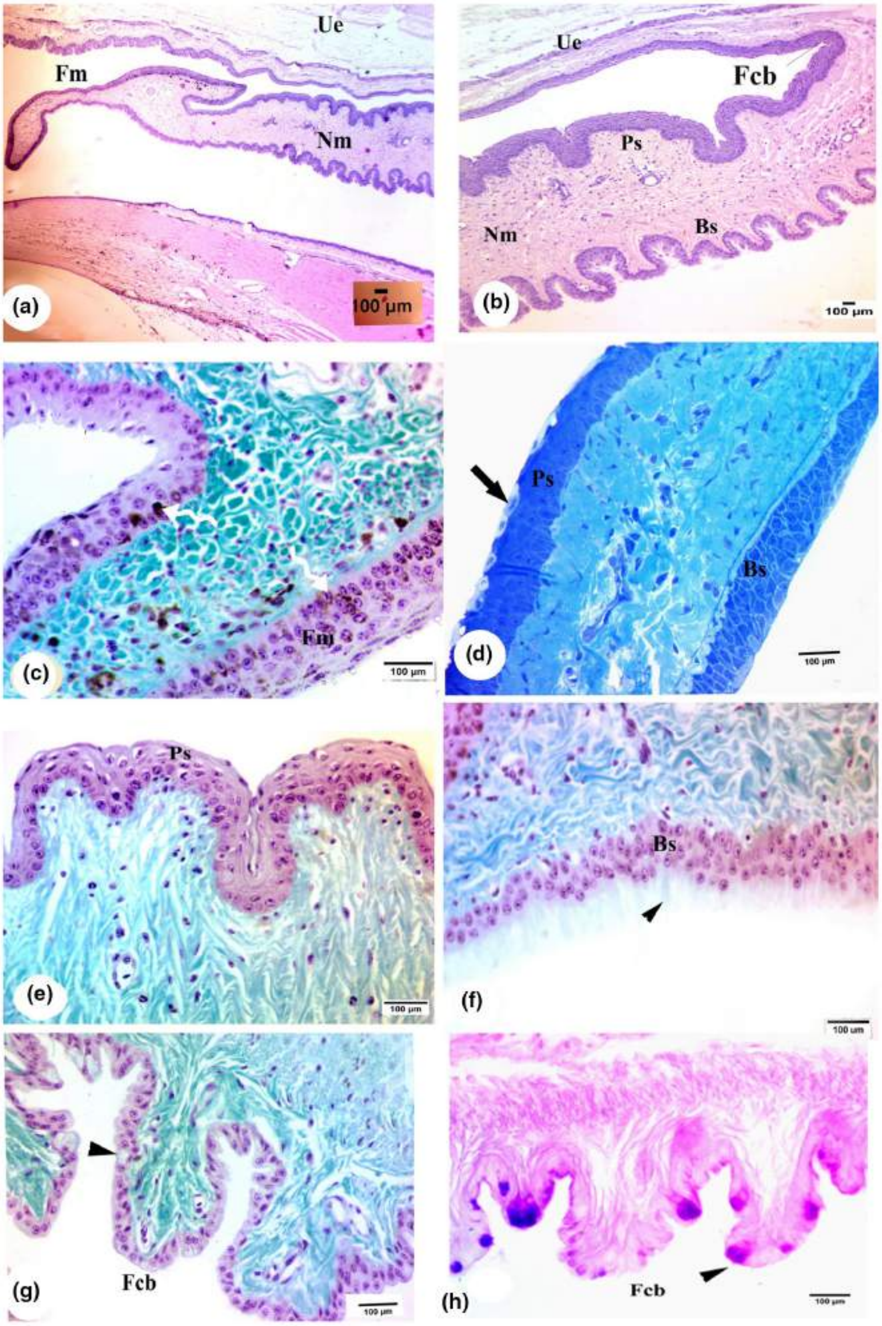

FIGURE 6 Photomicrograph of a transverse section of the nictitating membrane of little owl, *Athene noctua*, showing: (a) the surfaces of nictitating membrane; the palpebral surface (Ps), bulbar surface (Bs) and the free margin (Fm). (H& E, scale bar 100 µm). (b) the palpebral (Ps) and bulbar surface (Bs) and fornix conjunctiva palpebrae (Fcb). (H&E, scale bar 100 µm). (c) the free margin of the nictitating membrane (Fm) and pigmented granules (zigzag arrow). (Masson's trichromic, scale bar 100 µm). (d) the palpebral surface (Ps) with superficial cells has pale cytoplasm (arrow), bulbar surface (Bs; toluidine blue, scale bar 100 µm). (e) the palpebral surface (Ps) of the nictitating membrane (Masson's trichromic, scale bar 100 µm). (f) the bulbi surface (Bs) with long cytoplasmic extension like the feather duster (arrow head). (Masson's trichromic, scale bar 100 µm) (g and h) the epithelial of fornix conjunctiva bulbi (Fcb) with numerous unicellular mucous glands (arrow head) which exhibits positive reaction with PAS (g by Masson's trichromic, h by PAS, scale bar 100 µm).

3.9 | Retractor anguli oculi medialis muscle

Retractor anguli oculi medialis is a ribbon-like and parallel-fibred muscle that extends laterally from the anterior angle of the brain case to run ventrally, reaching the anterior angle of the eyelid. Along the extension of this muscle overpass is the nasolacrimal sac. The muscle retractor anguli oculi medialis originates from the medial surface of the lacrimal bone then extends antero-ventral towards its insert. The insertion of the retractor anguli oculi medialis is diverged; some fibres are attached onto the anterodorsal surface of the upper palpebrae margin while other fibres reach to the lower eyelids and spread fanning out on the inferior tarsal plate.

3.10 | Retractor anguli oculi lateralis muscle

Retractor anguli oculi lateralis is sheet-like with parallel-fibred muscle that extends from the posterior angle of the brain case to be attached to the posterior angle of the eyelids. The muscle retractor anguli oculi lateralis originates from the posterior rim of the orbit (anterior temporal crest) via fleshy fibres and aponeurosis which overpasses

FIGURE 7 Photo of sequence of video recording of movement of the nictitating membrane of little owl, *Athene noctua*, showing this membrane slips rapidly and obliquely across the front of the eye from a fixed point towards the ventrotemporal direction.

and is covered partially by the dorsal surface of the muscle levator anguli oculi. The origin site of the muscle retractor anguli oculi lateralis is located posterior and anterolateral to the origin site of levator anguli oculi and dermotemporalis muscle, respectively. The insertion of the retractor anguli oculi lateralis attaches onto the posterior angle of the upper eyelid and few muscle fibres and diverges to attach onto the posterolateral surface of the inferior tarsus plate.

3.11 | Depressor palpebrae inferioris muscle

The muscle of the depressor palpebrae inferioris is fan-shaped and parallel-fibred muscle lies dorsal to the muscle obliqus lateralis and rectus ventralis. The ventral surface of this muscle is covered partially by the periorbital sheet. The depressor palpebrae inferioris extends from the origin that lies on the interorbital septum (orbitosphenoid) just ventral to the optic foramen, medial to basiopterygoid process, and then fans out towards its insertion that is located on the posterior margin of the inferior tarsal plate.

3.12 | Quadrates muscle

The quadrates muscle is broad, sheet- and fan-like muscle located transversally dorsal to the optic nerve spread over most of the dorsal surface of the posterior segment of the eyeball. The quadrates muscle originate from the postero- dorsal edge of the sclera cartilage ventral to the insertion site of the rectus dorsalis and oblique dorsalis

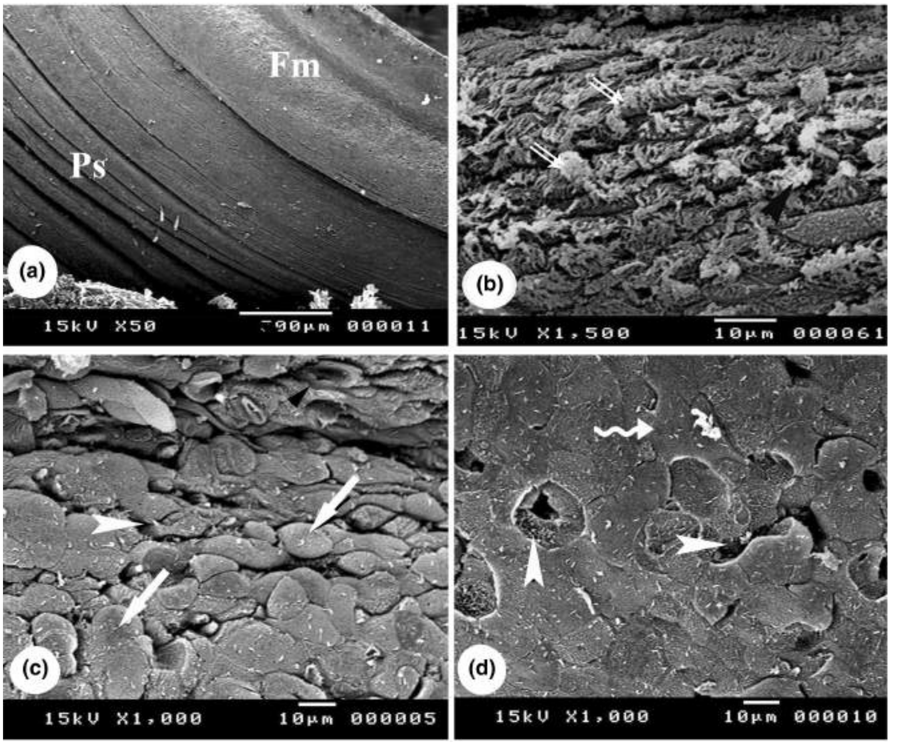

FIGURE 8 Scanning electron micrograph of the nictitating membrane of eye of little owl, *Athene noctua*, showing: (a) the membrane becomes more wrinkled medially than its anterior and posterior fixed point at anterior and posterior canthus. (b) the long cytoplasmic extension as the feather duster on the bulbi surface (double arrows). (c and d) the bulbi surface of the membrane have dome-shaped cells (arrow) becomes more flattened towards fornix conjunctiva (zigzag arrow), interspersed with scattered pores (arrow head.).

muscles. The muscle fibres extend posteriorly to be inserted via a thin aponeurosis which is on the tendon of the pyramidalis.

3.13 | Pyramidalis muscle

The muscle pyramidalis is triangle-shaped with parallel fibres, lies ventral to the rectus ventralis muscle. The muscle pyramidalis is located on the anteroventral surface of the sclera cartilage, and then extends posteriorly towards the optic nerve as a tendon. This tendon runs postero-ventrally then antero-dorsally towards the nictitating membrane to attach on the anterior edge of its free margin.

4 | DISCUSSION

The eyelids are unique protection tissues which extend from the skin to cover the eye socket when required. The eyelid movement is made involuntarily to protect the eye from the strong light or any foreign bodies, as well as keep the cornea surface moist (Jochems & Phillips, 2015). The presence of three eyelids, upper, lower, and third eyelid which is also known as the nictitating membrane, is a common feature in birds (Baumel, 1993). Together with the orbital glands, the eyelids supply the moisture for the eye and maintains their health (Klećkowska-Nawrot et al., 2016).

The movement of the eyelids of the little owl was recorded in the laboratory, and observed. The upper eyelid moves rapidly and frequently down towards the lower eyelid, while the slipping of the nictitating membrane occurs when the movement of the eyelids is fixed. The nictitating membrane slips in ventro-temporal direction. The movement of the upper eyelid in little owl is opposite to the views of most previous authors who confirmed that the lower eyelid of birds is more mobile than the upper one. That feature is common in mammals; the upper eyelid is more mobile and larger than the lower eyelid (Klećkowska-Nawrot et al., 2019).

The authors supposed that the little owl is one of the owl species that hunts during the day, waiting and

FIGURE 9 Photo of dissection of the eyelids muscles of little owl, *Athene noctua*, showing: the periorbital sheet (Psh), lacrimal process (Lp), the lacrimal gland (LG) retractor anguli oculi lateralis muscle (Raol), retractor anguli oculi medialis muscle (Raom), levator anguli oculi muscle (lao), dermatotemporalis muscle (Dt), lateral rectus muscle (Lr), aponeurosis of lateral rectus muscle (ap.Lr), ventral rectus muscle (Vr) and aponeurosis of ventral oblique muscle (Ap.Vo) which covers the harderian gland (HG), quadratus muscle (Qa), prymidalis muscle (py), prymidalis tendon (pyt), aponeurosis of dorsal rectus muscle (Ap.Dr), aponeurosis of dorsal oblique muscle (Ap. Do), the origin site of levator palpebrae superioris muscle (O.Lps) and depressor palpebrae inferioris muscle (O.dpi), retractor anguli oculi Lateralis muscle (O.Raol), retractor anguli oculi medialis muscle (O.Raom).

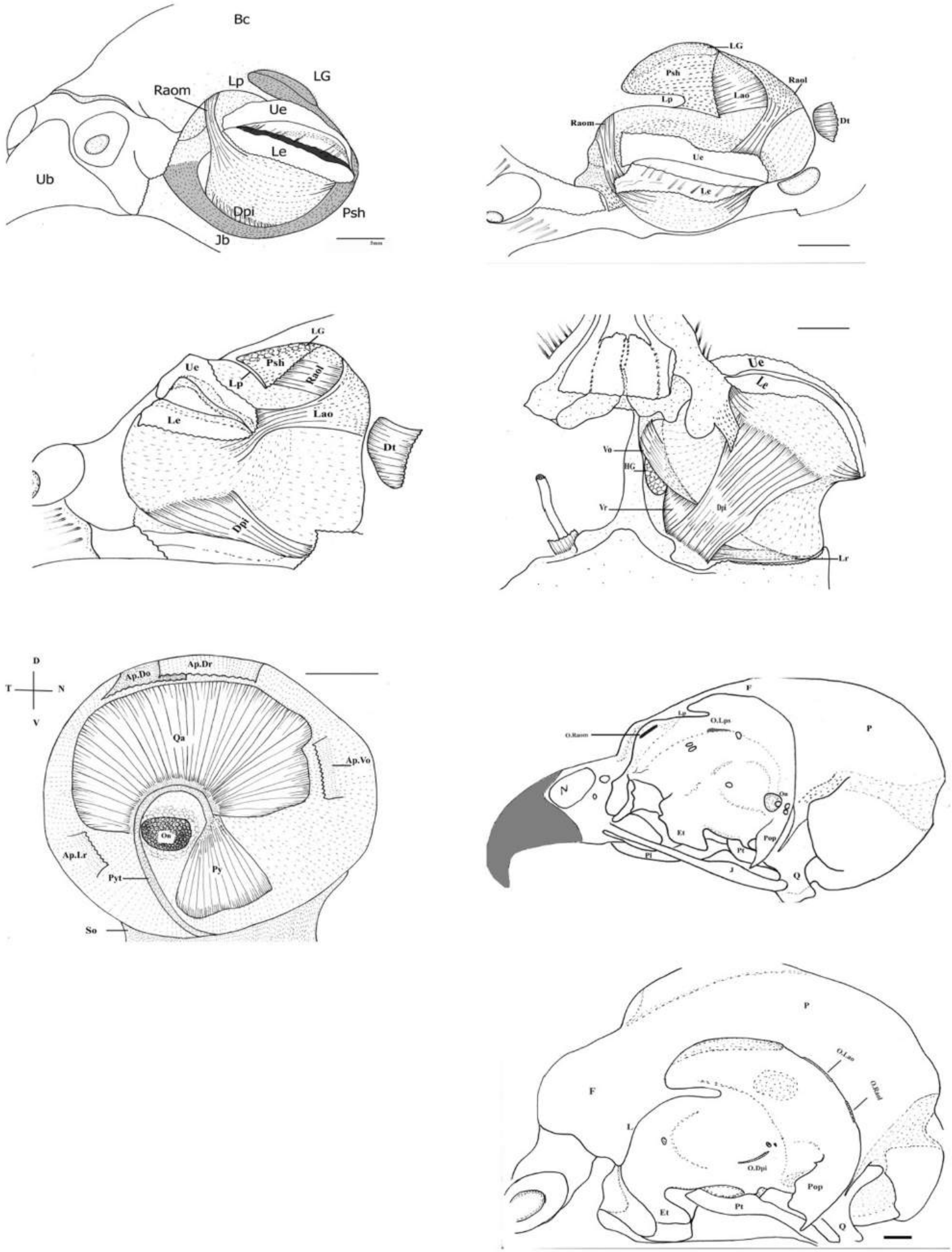
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TABLE 1 Summary of the origin, insertion, and function of the eyelid's muscles of little owl, *Athene noctua*

Name of muscle	Origin	Insertion	Function
Levator palpebral superioris muscle (Lps)	Fleshy, on the interorbital septum	On the connective tissue of the upper eyelid and nictitating membrane	The contraction of Lps muscle yields to elevate the upper eyelid, consequently it helps in the opening of the upper eyelid of the eye
Levator anguli oculi muscle (Lao)	Aponeurotic and fleshy, from anterior temporal crest	Fleshy, on the connective tissue of the upper eyelid and nictitating membrane	The muscle Lao cooperates in elevation of the upper eyelid and nictitating membrane by pulling their dense connective tissue backward. It is synergistic with Lps muscle
Retractor anguli oculi medialis muscle (Raom)	Fleshy, medial surface of the lacrimal bone	Fleshy, on the anterior angle of the eyelids	The muscle Raom closes the palpebral fissure by pulling the anterior angle of eyelids
Retractor anguli oculi lateralis muscle (Raol)	Aponeurotic, fleshy, from the anterior temporal crest	Fleshy, on the posterior angle of eyelids	The muscle Raol closes the palpebral fissure by pulling the posterior angle of the eyelids
Depressor palpebral inferioris muscle (Dpi)	Fleshy, from the interorbital septum (orbitosphenoid)	Fleshy, on the inferior tarsal plate	the contraction of the muscle Dpi yielding to depressing of the lower eyelid resulting in opening the palpebral fissure
Quadrates muscle (Qa)	Fleshy, from the dorsal surface of the sclera cartilage	Aponeurotic, on the tendon of pyramidalis	The contraction of Qa muscle draws the nictitating membrane posteriorly over the cornea of the eye, whereas relaxation allows the nictitating membrane to return to its resting position along the anterior edge of the eye
Pyramidalis muscle (Py)	Fleshy, from the antero-ventral side of the eyeball	Fleshy, on the tendon of pyramidalis	The muscle Py aids in closure of the nictitating membrane, the involvement of both muscles, with the sling and tendon mechanism, maintains a consistent direction of pull on the pyramidalis tendon regardless of the orientation of the eye

focusing on their prey and then captures them with sharp claws (Mikkola, 2014). The nature of the protective apparatus may be expected to vary according to its activity pattern. The little owl being mostly less near the ground, thereby less exposed to dust and dirt. It is may expected that this owl does not need any special mechanism to remove foreign bodies. The nictitation happens to cleanse the front of the cornea but in little owl, and this function is performed by the upper eyelid. Chauveau-Arloing (2018) pointed out the size of the nictitating membrane as inversely proportional to the ability of the animal to remove foreign bodies from its own eyes.

Jochems and Phillips (2015) said that the eyelid movement is made involuntarily as a reflex action against any foreign bodies. The authors agree with this view but suggested there are some conditions that may stimulate the movement of eyelids. The anatomical results showed that the whole eyeball with their muscles is surrounded by a collagenous periorbital sheet. The variations in the thickness of the periorbital sheet and the difference in its connections with the skull among vertebrates may reflect how much the importance of this sheet is. It was noticed that there is no available information about the importance of the periorbital sheet.

In little owl, this periorbital sheet lies directly beneath the epithelium of the eyelids and is covered by the levator and depressor muscles that control the movement of the upper and lower eyelids, respectively. Furthermore, this sheet in little owl connects with some movable skeletal parts of the skull, so any change in the movement of these skeletal parts during the feeding process will pull the periorbital sheet. Physically, the morphological features and topographical position of the periorbital sheet of the little owl allow making a pressure force on the levator and depressor eyelids muscles that may stimulate the contraction of these muscles, thereby causing the closing or opening of eyelids. Theoretically, we concluded that the eyelid movement depends not only on the periorbital sheet but also on the movement of the skeletal elements of the skull during feeding. Ostheim et al. (2020) studied the eyelid squinting during food pecking in pigeons. It was confirmed that the feeding and sensory systems are integrated systems. The present authors suggest that the periorbital sheet may play an important role in coordinating the movement of the eyelids.

Moreover, during anatomical investigation of the eye of little owl, we observed the orbital region (and the various glands therein) is connected with the nasal region nasolacrimal duct (tear duct). This duct appears in the little owl at anterior in oblique position, near the nictitating membrane. The retractor anguli oculi medialis muscle passes over this duct during its ventral extension to attach onto the lower eyelid. The retractor anguli oculi medialis muscle in the little owl is considered as one of accessory eye muscles that shares in closing the palpebral fissure by pulling the anterior angle of the lower eyelid. The role of this muscle was mentioned by Slonaker (1918) in English sparrow, Burk (1893) in pigeon, and Stibbe (1928) in mammals.

According to the anatomical feature of this muscle in little owl, the present authors expected that the contraction of the retractor anguli oculi medialis muscle may cause pressure on the nasolacrimal duct that may help it to perform its function in draining the orbital fluid.

However, the histological structure of eyelids facilitates its sweeping motion without any injury. The present study reveals that the structure of two eyelids is very similar in the little owl. Histologically, the clear variation between the two eyelids noticed among the number of cell layers forming the epithelium of skin and palpebral surfaces of the eyelids, the degree of keratinization over their surface, as well as the distribution of pigment granules within their cells. The skin surface of both eyelids of little owl is composed of keratinized squamous epithelium. The cell-layer of the skin surface varies from one to two layers in the lower eyelid while it reached approximately three to four layers in the upper eyelid. Thus, the lower eyelid in little owl appears very thin more than the upper lid. The keratin layers that cover their eyelids act as a protective armour. Kardong (2009) said that the keratinization occurs where friction or direct mechanical abrasion insult the epithelium.

The present investigation has observed that the margins of eyelids and its conjunctiva surface are non-keratinized, except a small area of the conjunctival surface of the lower eyelid, just near its palpebral margin. In 1979, Edward Moumenea has reported about the presence of keratinization of the conjunctiva and he assumed that the degree of keratinization probably does not depend on direct stimulation or initiation of keratin synthesis, but depends on the maintenance of organized basal layer; also, the keratinized cells produce an epithelial keratitis with subsequent vascularization of the cornea.

The surface of cells of the conjunctiva epithelium has carried a tiny microvilli "cytoplasmic extension" and contains mucous goblet cells. The goblet cells appear in the upper eyelid in large numbers in comparison to those in the lower one. A similar result has been described in Barred owl, *Strix varia* (Jochems & Phillips, 2015). It was found that these cytoplasmic extensions are seen also on the bulbar surface of the third eyelid. Schramm et al. (1994) pointed out that the densely clustered microvilli exhibit resorptive capacity towards substances with low molecular weights.

The histochemical analysis of the glands that are scattered within the palpebral and bulbi conjunctiva showed that their secretions are mucous neutral and acid substances. These secretions are distributed over the corneal surface by the movement of eyelids and by aiding the tiny microvilli to protect the corneal surface from any foreign body and keep it moist. Furthermore, the acidic nature for these glands affords immunoprotection to the cornea.

These protective and cleaning functions are supplemented by the gliding movement of the nictitating membrane. The nictitating membrane is a highly specialized neuro-muscular system (Stibbe, 1928). Previous studies have found that the nictitating membrane appears more prominent and well developed in the eye of some vertebrates, for example, birds and reptiles, as well as many mammals which are exposed to wind, storm, dust, and sand (Schramm et al., 1994).

During the movement of the nictitating membrane crosses over the cornea surface may expose the cornea surface to great friction, resulting in loss of optical quality. The anterior corneal surface of the little owl is covered by a multilayer of cuboidal cells that proliferate superficially to form a disc of flattened cells. This type of cornea epithelium may counteract the abrasive forces which occur in this region during the sweeping process of this membrane. Simultaneously, the fluid that is secreted from the orbital glands moistens this membrane and helps to decrease the abrasive force and facilitate its sweeping motion without any injury.

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