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From Cell Biology to Tissue Engineering

Comparative study of the eyelids and orbital glands morphology in the okapi (*Okapia johnstoni*, Giraffidae), Père David's deer (*Elaphurus davidianus*, Cervidae) and the Philippine mouse-deer (*Tragulus nigricans*, Tragulidae)

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Summary. The accessory organs of the eye represent part of the protective system of the eyeball. In the present study, an examination of the accessory organs of the eye of three species of captive ruminants was performed using light microscopy. In the okapi, the superficial gland of the third eyelid and lacrimal gland were complex branched multilobar tubular glands formed by mucous units with tubular secretory portions and no plasma cells. The deep gland of the third eyelid was absent in the okapi and present in both the Père David's deer and the Philippine mouse-deer. In the Philippine mouse-deer, the deep gland had a very thick connective capsule and thick interlobar septae. It contained fewer lobes forming the gland parenchyma compared to Père David's deer and other ruminants. Organized lymphoid follicles were present within the upper and lower eyelids only in the okapi and Père David's deer, while diffuse lymphocytes were observed in the Philippine mouse-deer. The orbital glands in the Père David's deer had a multilobar tubuloacinar structure with numerous plasma cells and a mucoserous character. In contrast to the Philippine mouse-deer, these glands had a serous character. The presence of several macroscopic and microscopic structural differences of the examined accessory organs of the eye in the three captive ruminant species may be understood within an ecological context and may be associated with different habitat-specific environmental conditions.

Key words: Morphology, Histology, Histochemistry, Accessory organs of the eye, Ruminants

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Introduction

The upper and lower eyelid, the superficial gland of the third eyelid together with the third eyelid, the deep gland of the third eyelid and the lacrimal gland are accessory organs of the eye (*organa oculi accessoria*) (Nomina Anatomica Veterinaria, 2017).

The upper and lower eyelids are cutaneomuscular folds surrounding the palpebral fissure. Both eyelids have a characteristic histological structure and house numerous glands (Fig. 1). The main function of the eyelids is to protect the eyeball from trauma, maintain a moist ocular surface, and regulate the amount of light reaching the eye. In addition, eyelids distribute tear film over the cornea (Yasui et al., 2006; Gasser et al., 2011; Voight et al., 2012).

The conjunctiva-associated lymphoid tissue (CALT), which is part of the eye-associated lymphoid tissue

(EALT), is closely associated with the eyelids. The CALT and the deep gland of the third eyelid (in species that contain this gland) form the head-associated lymphoid tissue (HALT) (van Ginkel et al., 2012; Nasrin et al., 2013). The CALT is part of the eye-associated lymphoid tissue (EALT) (Knop and Knop, 2000). The EALT appendage forms lacrimal gland-associated lymphoid tissue that covers the conjunctiva and drains into the lacrimal drainage-associated lymphoid tissue (LDALT) (Knop and Knop, 2000, 2005). Histological study of the eyelids in Japanese monkey (Kageyama et al., 2006) and in ostrich (Bayraktaroglu et al., 2011) showed that there were differences in the conjunctival surface between the lymphoid conjunctiva and non-lymphoid conjunctiva, also known as the lymphoid region and non-lymphoid region. The lymphoid conjunctiva was characterised by a lack of goblet cells in the epithelium and aggregated and solitary lymphoid follicles, while the non-lymphoid region was covered by a stratified columnar epithelium containing goblet cells. Immunophenotyping of lymphocytes in the canine third eyelid showed the presence of three patterns concerning the immunophenotype of the third eyelid tissue (Alexandre-Pires et al., 2008).

The orbital glands in mammals are classified into two groups of major exocrine glands (Aldana Marcos et al., 2002; Rehorek et al., 2010). One group includes glands located in the inner canthus of the orbit. These are the deep gland of the third eyelid, also known as the Harderian gland, and the superficial gland of the third eyelid, also called the nictitans gland (Sakai, 1992; Payne, 1994; Aldana Marcos et al., 2002; Klećkowska-Nawrot et al., 2015a; Klećkowska-Nawrot et al. 2016). The second group includes the lacrimal gland, which is located in the outer canthus of the orbit (Sakai, 1992; Aldana Marcos et al., 2002; Klećkowska-Nawrot et al., 2013, 2015b).

The superficial gland of the third eyelid is generally a compound multilobar tubuloacinar gland that produces a mucoserous secretion. This secretion forms the mucous fraction of the precorneal tear film, which lubricates and protects the cornea, prevents desiccation and bacterial contamination and anchors the aqueous tear film to the corneal epithelium protecting it from shear forces (McKenzie et al., 2000; Davidson and Kuonen, 2004). The superficial gland of the third eyelid that surrounds the third eyelid was found in Artiodactyla, Carnivora, Insectivora, Lagomorpha, Marsupials, Perissodactyla and Primates (Sakai, 1981; Sakai and van Lennep, 1984; Aldana Marcos et al., 2002). The third eyelid provides mechanical protection of the cornea and local immunological protection by means of lymph nodules and diffuse lymphocytes. It also distributes precorneal tear film on the corneal surface (Lavach, 1990; Schlegel et al., 2003; Klećkowska-Nawrot et al., 2015b).

The deep gland of the third eyelid of many mammals is located on the anterior surface of the orbit and consists of multilobar tubuloacinar glands, which are larger than the lacrimal gland and the superficial gland of the third eyelid. However, the anatomical location, size and morphological details of the deep gland of the third eyelid vary across mammalian species (Aldana Marcos and Affani, 2005; Rehorek et al., 2007; Klećkowska-Nawrot et al., 2015b). The main function of the deep gland of the third eyelid in mammals is to produce secretions that form the precorneal tear film, which lubricates and protects the eyeball and the third eyelid. In rodents, the deep gland of the third eyelid plays a photoprotective role and forms part of a photoreceptor in the retinal-pineal axis and light-pineal-gonadal axis. The deep gland of the third eyelid is also reported to be a source of pheromones, hormones, growth factors, thermoregulatory lipids, and factors for corneal repair and maintenance (Thiessen and Kittrell, 1980; Djeridane, 1992).

To date, there have been several studies of the lacrimal gland in domestic and wild mammals (Obata, 2006; Rehorek et al., 2007, 2010; Klećkowska-Nawrot and Dzięgiel, 2008; Ding et al., 2010; Schechter et al., 2010; Abbasi et al., 2014). The lacrimal gland is generally a multilobar tubuloacinar gland, which produces a serous and mucoserous secretion in Artiodactyla, Carnivora, Insectivora, Lagomorpha, Perissodactyla, Primates and Rodentia (Sakai, 1989; Aldana Marcos et al., 2002; Pinard et al., 2003a; Alsafy, 2010; Klećkowska-Nawrot et al,. 2015b).

Much research has been conducted on rodent orbital glands (e.g. Payne, 1994), but less is known about larger mammals, e.g. Artiodactyla (see Table 1). To date, the macroscopic and microscopic structure of the eyelids and orbital glands in the okapi (*Okapia johnstoni*, Giraffidae), Père David's deer (*Elaphurus davidianus*, Cervidae) and Philippine mouse-deer (*Tragulus nigricans*, Tragulidae) has not been described in detail. There are few studies on the morphology of these organs

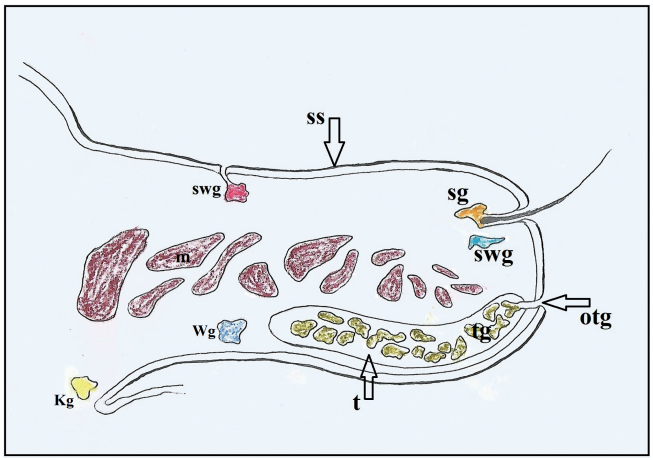

Fig. 1. Graphical structure of the eyelid based on Stewens and Lowe, 1994. Kg, Krause's gland; otg, opening of the tarsal glands; m, muscle fibres; sg, sebaceous gland; ss, skin surface; swg, sweat gland; t, tarsus; tg, tarsal glands; Wg, Wolfring's gland.

in captive ruminants, particularly of the species that are considered endangered (EN), according to the IUCN Red List of Threatened Species (2016), such as the okapi and Philippine mouse-deer, or critically endangered (CR) and listed under criterion "D" of the IUCN Red List of Threatened Species (2016) and deemed extinct in the wild (EW). Therefore, the aim of this study was to perform a comparative analysis of chosen structures in these three captive ruminant species living under natural conditions. The conducted study complements the current knowledge of the comparative ocular anatomy in domesticated and wild animals.

Materials and methods

Animals

The study was conducted on three species of captive ruminants (five adult animals): one male okapi (*Okapia johnstoni*, Sclater, 1901) (Giraffidae), one female Père David's deer (*Elaphurus davidianus*, Milne-Edwards, 1866) (Cervidae) and three female Philippine mouse-deer (*Tragulus nigricans*, Thomas, 1892) (Tragulidae). The study samples were collected from 2015 to 2017. All the animals were obtained by the Wroclaw Zoological Garden (Poland). The study was carried out with the permission of the District Veterinary Officer (Wroclaw, Poland; No. PIW Wroc. UT-45/5/16, No. PIW Wroc. UT- 45/6/16, No. PIW Wroc. UT-45/8/16). The animals were not killed for the purpose of this study and were obtained *post-mortem*. According to Polish and European law, studies on tissues obtained *post-mortem* do not require an approval of the Ethics Committee (Official Journal of the European Union L276/33: Directive of the European Parliament and of the Council 2010/63/UE from 22 September 2010 concerning the protection of animals used for experimental and other scientific purposes, and the Journal of Laws of the Republic of Poland Act on the Protection of Animals Used for Scientific or Educational Purposes from January 15th, 2015). The left and the right eyeballs and the accessory organs of the eye together with the left and the right upper, lower and third eyelid were collected immediately after the natural death of the animals. They were then fixed in 4% phosphate buffered formaldehyde for 72 hours.

Macroscopic study

The orbital glands and eyelids were first examined with a Zeiss Stemi 2000-C stereoscopic microscope (Carl Zeiss, Jena, Germany). The morphometric studies were performed on the left and right eyelid and orbital gland samples collected from all the animals. The morphometric measurements of the organs were conducted in all the collected glands using an electronic slide caliper with an accuracy of 0.1 mm. The values of six random measurements of length, width and thickness each of all the collected glands were analysed statistically and expressed as mean ± standard deviation (SD).

The terminology used in this manuscript is in accordance with the prevailing veterinary nomenclature

Table 1. Review of the presence of superficial gland of the third eyelid, deep gland of the third eyelid, third eyelid and lacrimal gland of the selected Artiodactyla.

Species family	SGTE	DGTE	TE	LG	Reference
European bison (Bison bonasus bonasus, Bovidae)	+	+	+ "anchor" shape	+	Klećkowska-Nawrot el al., 2015b
Alpaca (Vicugna pacos, Camelidae)	+	-	+ "anchor" shape	+	Klećkowska-Nawrot el al., 2015a
Red deer doe (Cervus elaphus, Cervidae)	N/A	+	N/A	N/A	Hraste et al., 1996
Roe deer doe (Capreolus capreolus, Cervidae)	+	+	N/A	+	Hraste et al., 1996; Klećkowska-Nawrot et al., 2008b, 2013
Sheep (Ovis aries, Bovidae)	+	+	+ "T" shape	+	Abbasi et al., 2014; Ceccareli et al., 1998; Dyce at al., 1996; Gargiulo et al., 1999; Kühnel, 1968c; Schlegel et al., 2001; Smythe, 1958
Goat (Capra hircus, Bovidae)	+	+	+ "T" shape	+	Das Shina and Calhoun, 1966; Dyce at al., 1996; Kühnel, 1968b; Schlegel et al., 2001; Smythe, 1958
American bison (Bison bonasus, Bovidae)	+	+	N/A	+	Pinard et al., 2003b
Fallow deer (Dama dama, Cervidae)	+	+	+	N/A	Rehorek et al., 2007
Chinese muntjac (Muntiacus reevesi, Cervidae)	+	+	+	N/A	Rehorek et al., 2007
Dromedary or Arabian camel (Camelus dromedaries, Camelidae)	+	+	+ "T" shape	+	Abuelhassan, 1999; Al-Ramadan and Ali, 2012; Alsafy, 2010; Fahmy et al., 1971; Ibrahim and Abdalla, 2015; Ibrahim, 1990; Mohammadpour, 2008
Buffalo (Bubalus bubalis, Bovidae)	+	+	+ "T" shape	+	Girgiri and Kumar, 2018; Maala et al., 2007; Salman, 2016; Shadkhast and Bigham, 2010
Cattle (Bos taurus taurus, Bovidae)	+	+	+ "anchor" shape	+	Dyce et al., 1996; Kühnel, 1968a; Pinard et al., 2003b; Schlegel et al., 2001

SGTE, superficial gland of the third eyelid; DGTE, deep gland of the third eyelid; TE, third eyelid; LG, lacrimal gland; N/A, not analysed.

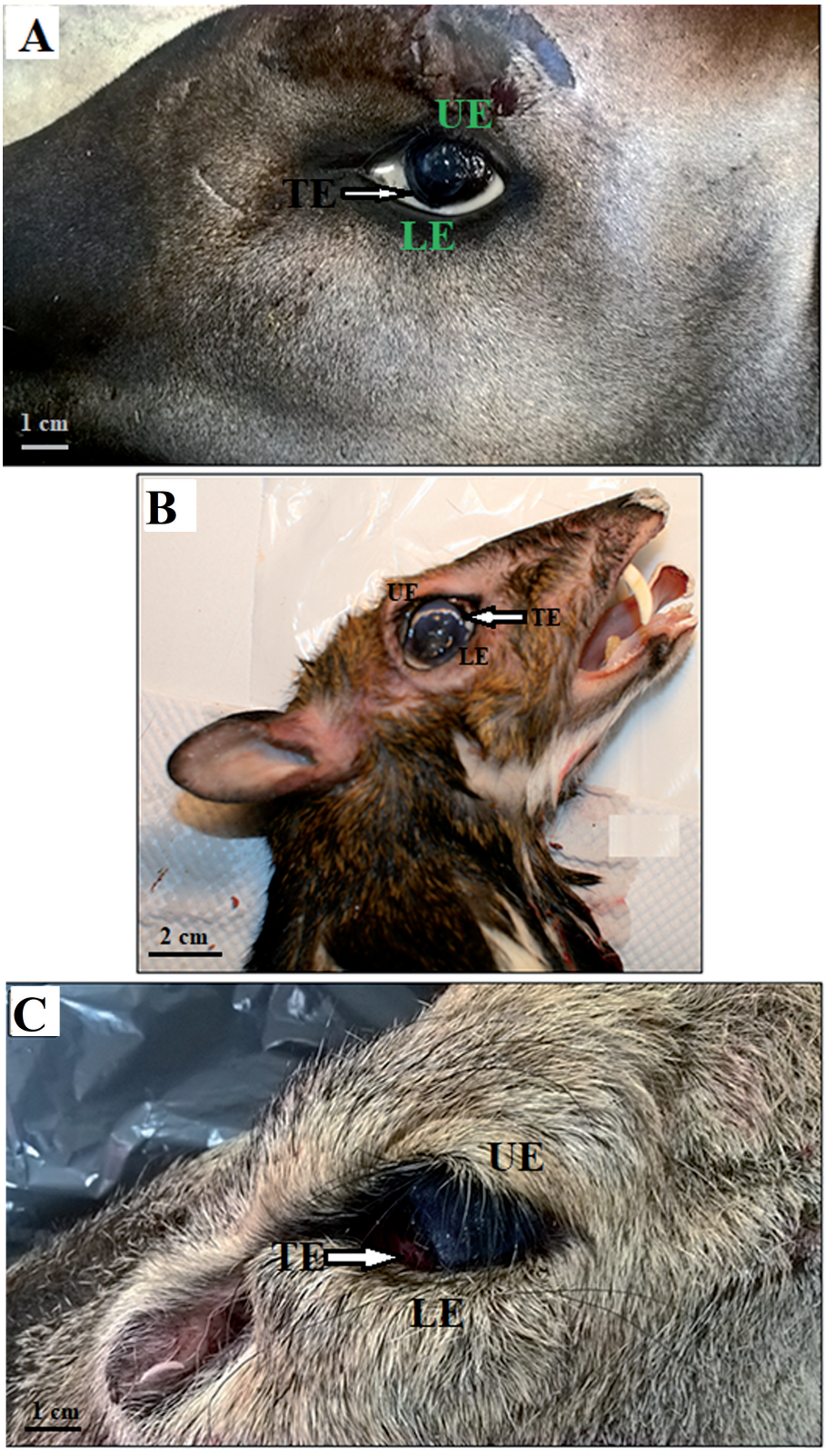

Fig. 2. Macrograph of the examined captive ruminants. **A.** Okapi. **B.** Philippine mouse-deer. **C.** Père David's deer.

(Nomina Anatomica Veterinaria, 2017).

Light microscopy examination

Histological findings

The samples were directly fixed in 4% buffered formaldehyde for at least 72 hours and washed in running water for 24 hours. The study samples were dehydrated through an ethanol series, and they were then processed in a ETP (RVG3, INTELSINT, Italy) vacuum tissue processor, embedded in paraffin and cut using a Slide 2003 (Pfm A.g., Germany) sliding microtome into 3-4 μm sections. The samples were divided into two groups: one that contained histological stainings and one for histochemical studies. Each structure (left and right) was cut either for histology and histochemistry. They were then stained with hematoxylin and eosin (general histological examination), azan trichrome (collagen and reticular fibers, muscle), Masson-Goldner trichrome (muscle, collagen fibers), Mallory trichrome (elastic fibers, collagen fibers, myofibrils) and Movat pentachrome (modified Russell Movat) (mucin, muscle, elastic, collagen and reticular fibers). The staining scoring system was based on a previously described standard protocol (Mallory, 1938; Movat, 1955; Burck, 1975; Lee, 1992).

In addition, the methyl green-pyronin Y (MGP Y) method (Kurnick, 1955) was used to detect the presence of plasma cells.

Histomorphometric study in hematoxylin and eosin stained sections of the orbital glands

In order to determine the capsule thickness and the interlobar septal thickness, the acinar outer diameters and the tubular outer diameters were measured according to the method described by El-Fadaly et al., (2014) and are presented in Table 2. The superficial and deep gland of the third eyelid and the lacrimal gland were measured. Histological measurements of the gland structure were carried out using the Axio Vision 4.8 (Carl Zeiss MicroImaging GmbH, Jena, Germany) software. Statistical analysis was performed using Prism 3.0 software (GraphPad Software, Inc. San Diego, USA). Data were analysed statistically and expressed as mean ± standard deviation (SD).

Histochemical analysis

The periodic acid-Schiff (PAS) method was used to identify glycans, glycoconjugates and neutral or weakly acidic glycoproteins (Sheehan and Hrapchak, 1980). The Alcian blue pH 2.5 method was used to identify sulphated and carboxylated acid mucopolysaccharides and sulphated and carboxylated sialomucins (glycoproteins) (Carson, 1990). Hale's dialysed iron staining (HDI) was carried out to determine the presence of sulphated acid mucosubstances (SAM) and carboxylated acid mucosubstances (CAM) (Spicer and Henson 1967; Munakata et al., 1985).

All the obtained slides were examined histologically and histochemically using the Zeiss Axio Scope A1 light microscope (Carl Zeiss, Jena, Germany).

Results

The upper and lower eyelids

Gross anatomy

As in other mammals, the upper eyelid of the examined ruminants was more mobile and larger than the lower eyelid. In the okapi and Philippine mouse-deer, there were long and thin eyelashes observed on the anterior palpebral margin of the upper eyelid (Fig. 2A,B). There were no eyelashes on the lower eyelid. In the Père David's deer, long and thick eyelashes were also observed on the anterior palpebral margin of the upper eyelid, whereas short and delicate eyelashes were present in the anterior palpebral margin of the lower eyelid (Fig. 2C).

Histological study

In the examined ruminants, the upper and lower eyelids consisted of an external skin surface and an internal conjunctival surface. The external skin surface of the eyelids was covered by a keratinized squamous epithelium with four to six layers of nucleated cells in the okapi (Fig. 3A), with six to 10 layers in the Père David's deer and with two to five layers in the Philippine mouse-deer. Scattered melanocytes were present in the stratum basale. The conjunctival surface of the upper and lower eyelids consisted of two parts known as the

Table 2. The measurement of the capsule and interlobar thickness, the acinar and tubular diameter of the superficial gland of the third eyelid, deep gland of the third eyelid and lacrimal gland according to the method described by El-Fadaly et al., (2014).

Measured structure	Method used
Capsule thickness	60 randomly chosen parts from five animals/both sides/gland; 50x magnification
Interlobar septal thickness	60 randomly chosen parts from five animals/both sides/gland; 50x magnification
Acinar and tubular outer diameters	widest diameter of the transversely cut acini and tubules from 60 randomly chosen parts from five animals (15 acini/animal/gland and 15 tubules/animal/gland) from both sides; 400x magnification

marginal zone and the orbital zone. The marginal zone formed by external border of the upper and lower eyelid, while the orbital zone included part of the conjunctiva that contacted the eyeball. The marginal zone of the conjunctival surface of the eyelids was covered by a stratified columnar epithelium with 15 to 20 layers of cells in the okapi, six to seven layers in Père David's deer (Fig. 3B) and five to seven layers of cells in Philippine mouse-deer. Six to seven non-keratinized layers of the orbital zone in the lymphoid region without goblet cells were present in the okapi, five to seven layers in Père David's deer and four to five layers of cells in the Philippine mouse-deer (Fig. 3C). The epithelium in the non-lymphoid region consisted of a single or double layer of squamous epithelial cells with numerous goblet cells in the okapi and the Père David's deer. However, single layers were present in the Philippine mouse-deer.

The superior tarsus of the upper eyelid and inferior tarsus of the lower eyelid consisted of dense fibrous connective tissue (Fig. 3D) and the thickest tarsus was found in the okapi (Table 3). The tarsal glands were present inside the superior and inferior tarsus. The tarsal glands in the examined ruminants were elongated and alveolar with ducts that were lined by a stratified squamous epithelium and openings in the posterior palpebral margins of both eyelids (Fig. 3D). Numerous sebaceous glands and sweat glands were located deep in the follicle and appeared as aggregations of three to 14 lobules of variable sizes that secreted sebum into the hair follicles in the okapi (Fig. 3E). Sebaceous glands were

Table 3. Morphometric parameters (μm) of the superior tarsal and inferior tarsal thickness in the okapi, the Père David's deer and the Philippine mouse-deer.

Species	Superior tarsal thickness (μm)	Inferior tarsal thickness (μm)
Okapi	635.75±89.1	407.76±36.9
Père David's deer	382.72±29.3	264.82±12.2
Philippine mouse-deer	312.18±10.01	201.29±7.03

Values are expressed as mean ± standard deviation.

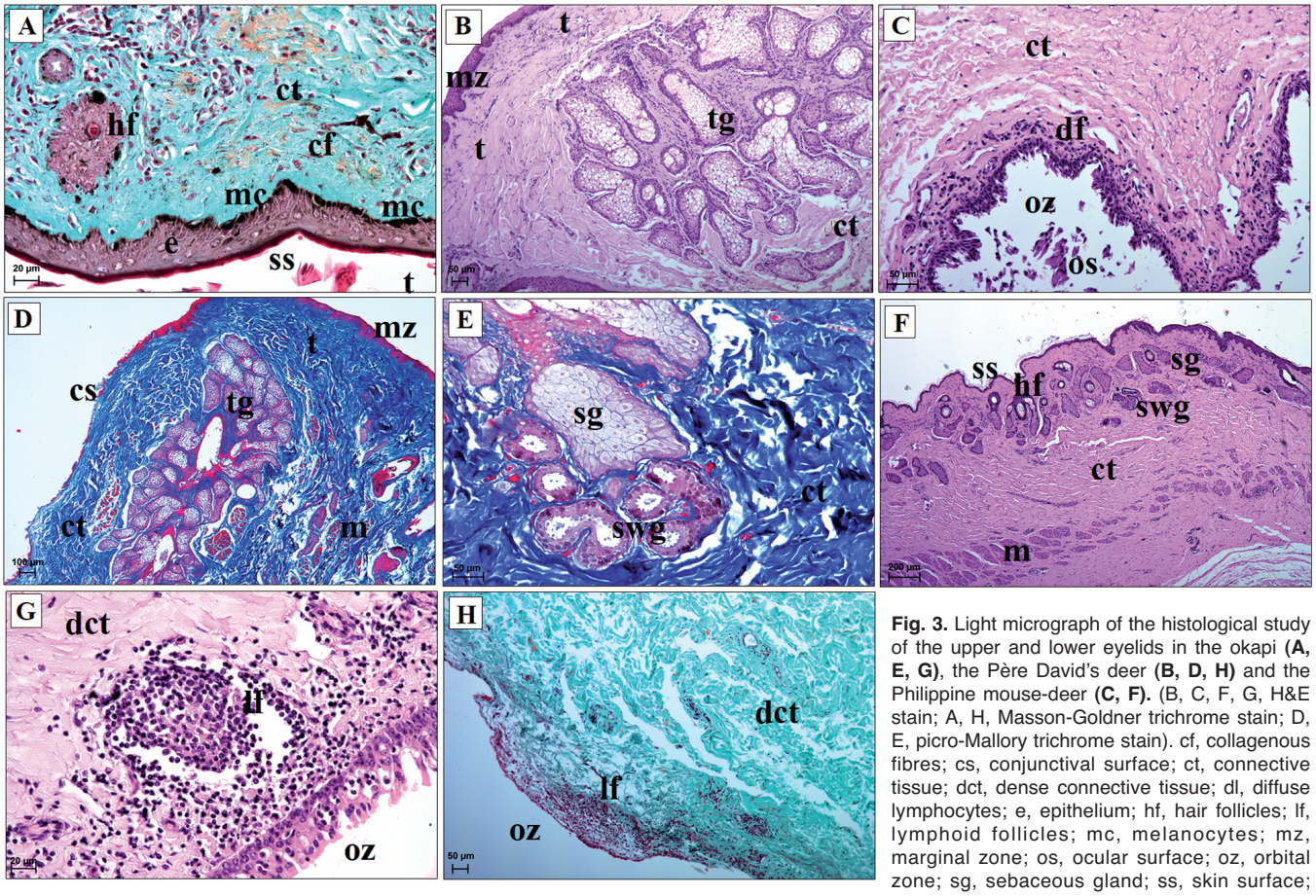

Fig. 3. Light micrograph of the histological study of the upper and lower eyelids in the okapi **(A, E, G)**, the Père David's deer **(B, D, H)** and the Philippine mouse-deer **(C, F).** (B, C, F, G, H&E stain; A, H, Masson-Goldner trichrome stain; D, E, picro-Mallory trichrome stain). cf, collagenous fibres; cs, conjunctival surface; ct, connective tissue; dct, dense connective tissue; dl, diffuse lymphocytes; e, epithelium; hf, hair follicles; lf, lymphoid follicles; mc, melanocytes; mz, marginal zone; os, ocular surface; oz, orbital zone; sg, sebaceous gland; ss, skin surface; swg, sweat gland; t, tarsus; tg, tarsal glands. Scale bars: A, G, 20 μm; B, C, E, H, 50 μm; D, 100 μm; F, 200 μm.

observed in okapi and Père David's deer only in upper eyelid. There were three to six lobules in the Père David's deer and three to five lobules in the Philippine mouse-deer. In the three examined ruminants, a thick layer of muscle, consisting of bundles of the orbicularis oculi muscle, the *levator anguli oculi medialis* and the malaris muscles, was located below the superior tarsus and inferior tarsus (Fig. 3F). Dense irregular connective tissue with a network of collagen and elastic fibers, numerous blood vessels and nerve fibers were found in the orbital zone.

Numerous lymphoid follicles and diffuse lymphatic cells forming the lymphoid region were located mainly in the lower eyelid in the okapi (Fig. 3G) and Père David's deer (Fig. 3H). On the other hand, single diffuse lymphatic cells formed the lymphoid region of the upper and lower eyelids in the Philippine mouse-deer (Fig. 3C). Additionally, no lymphoid follicles were present in the studied samples in either eyelid of the Philippine mouse-deer.

The palpebral part of the lacrimal gland in Père David's deer and Philippine mouse-deer were observed in the upper eyelid (Figs. 4A,B).

The third eyelid

Gross anatomy

In the studied animals, the third eyelid was located in the medial canthus of the eye. The marginal part of the third eyelid was pigmented and thick. The third eyelid in the Père David's deer and the Philippine mouse-deer was T-shaped. In the okapi, the third eyelid resembled the letter "Y", while the upper and lower branches resembled the letter „U" and were directed toward the temporal angle of the eye. The third eyelid in all the examined ruminants contained cartilage, which consisted of an upper and lower branch and a crossbar. The crossbar was covered and surrounded by the

Table 4. Morphometric parameters (mm) of the superficial gland of the third eyelid (SGTE), the deep gland of the third eyelid (DGTE) and lacrimal gland (LG) in the okapi, the Père David's deer and the Philippine mouse-deer.

Examined gland	Species	Length (mm)	Width (mm)	Thickness (mm)
SGTE	Okapi	23.74±1.9	26.91±1.5	8.71±0.4
	Père David's deer	19.03±0.3	17.61±0.4	7.75±0.3
	Philippine mouse-deer	7.77±0.1	6.62±0.3	3.72±0.2
DGTE	Okapi	N/A	N/A	N/A
	Père David's deer	25.96±1.1	22.11±1.09	16.86±0.6
	Philippine mouse-deer	9.36±0.3	9.63±0.2	4.87±0.1
LG	Okapi	34.44±0.8	18.21±0.6	4.54±0.6
	Père David's deer	41.32±0.7	19.03±0.5 10.78±0.2	4.85±0.2
	Philippine mouse deer	14.77±0.3	6.85±0.3	3.29±0.2

Values are expressed as mean ± standard deviations.

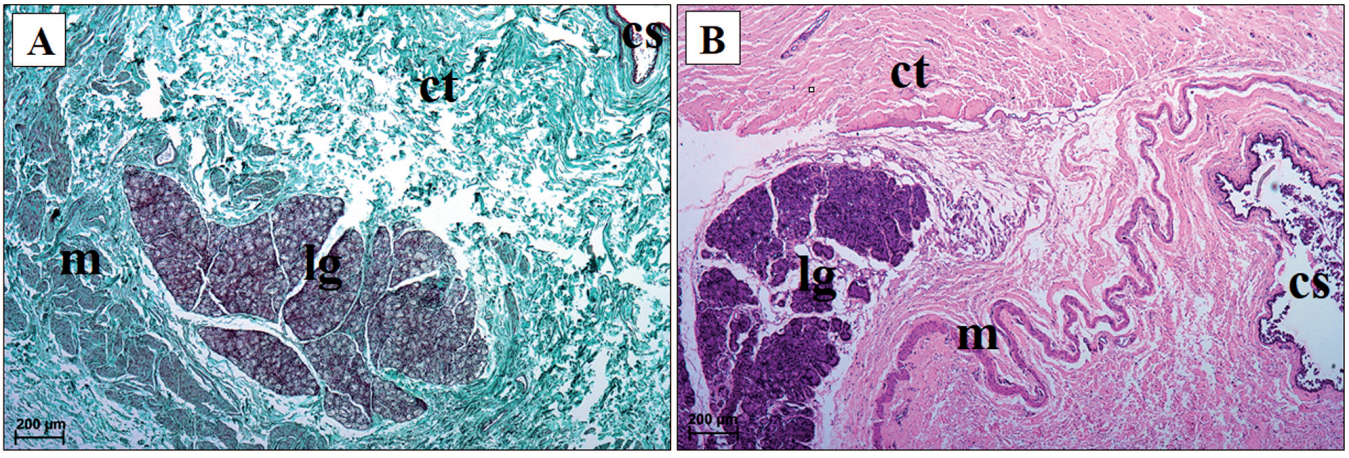

Fig. 4. Light micrograph of the histological study of the palpebral part of the lacrimal gland in Père David's deer **(A)** and the Philippine mouse-deer **(B).** (B, H&E stain; B, Masson-Goldner trichrome stain). cs, conjunctival surface; ct, connective tissue; lg, lacrimal gland - palpebral part; m, muscle. Scale bars: 200 μm.

superficial gland of the third eyelid and an intraperiorbital fat body.

Histological study

In the examined ruminants, the free margin of the third eyelid had a thick non-keratinized stratified squamous epithelium with numerous melanocytes (Fig. 5A). The connective tissue parenchyma of the third eyelid was composed of dense connective tissue with collagen and elastic fibres and numerous blood vessels (Fig. 5B). The ocular surface was covered by a non-keratinized stratified columnar epithelium with four to eight layers of cells in Père David's deer (Fig. 5C), five to 10 layers of epithelial cells and numerous goblet cells in the okapi (Fig. 5D), and three to five layers of cells in the Philippine mouse-deer. The conjunctival surface of the third eyelid was covered by a non-keratinized stratified squamous epithelium with five to eight layers of nucleated cells and goblet cells in the okapi, four to 10 layers in Père David's deer and three to five layers of cells in the Philippine mouse-deer (Fig. 5E).

In the okapi and Père David's deer, the lymphoid follicles were present in the lymphoid region of the ocular surface without goblet cells (Fig. 5F). On the other hand, diffuse lymphocytes were observed in the lamina propria of the subepithelium in the okapi and Philippine mouse-deer (Fig. 5G).

The third eyelid cartilage in the okapi and Philippine mouse-deer was surrounded by a thin layer of elastic and collagen fibres and additionally contained abundant adipose tissue in the Philippine mouse-deer (Fig. 5H). The cartilage in the examined ruminants was composed of hyaline tissue with single chondrocytes and abundant intercellular substance. In the superficial layers, the chondrocytes were small and flattened, while they were large and oval in the deeper parts of the eyelid. In the hyaline cartilage, isogenic groups of two to three chondrocytes or single chondrocytes were observed (Fig. 5E,H).

The superficial gland of the third eyelid

Gross anatomy

The superficial gland of the third eyelid in all the

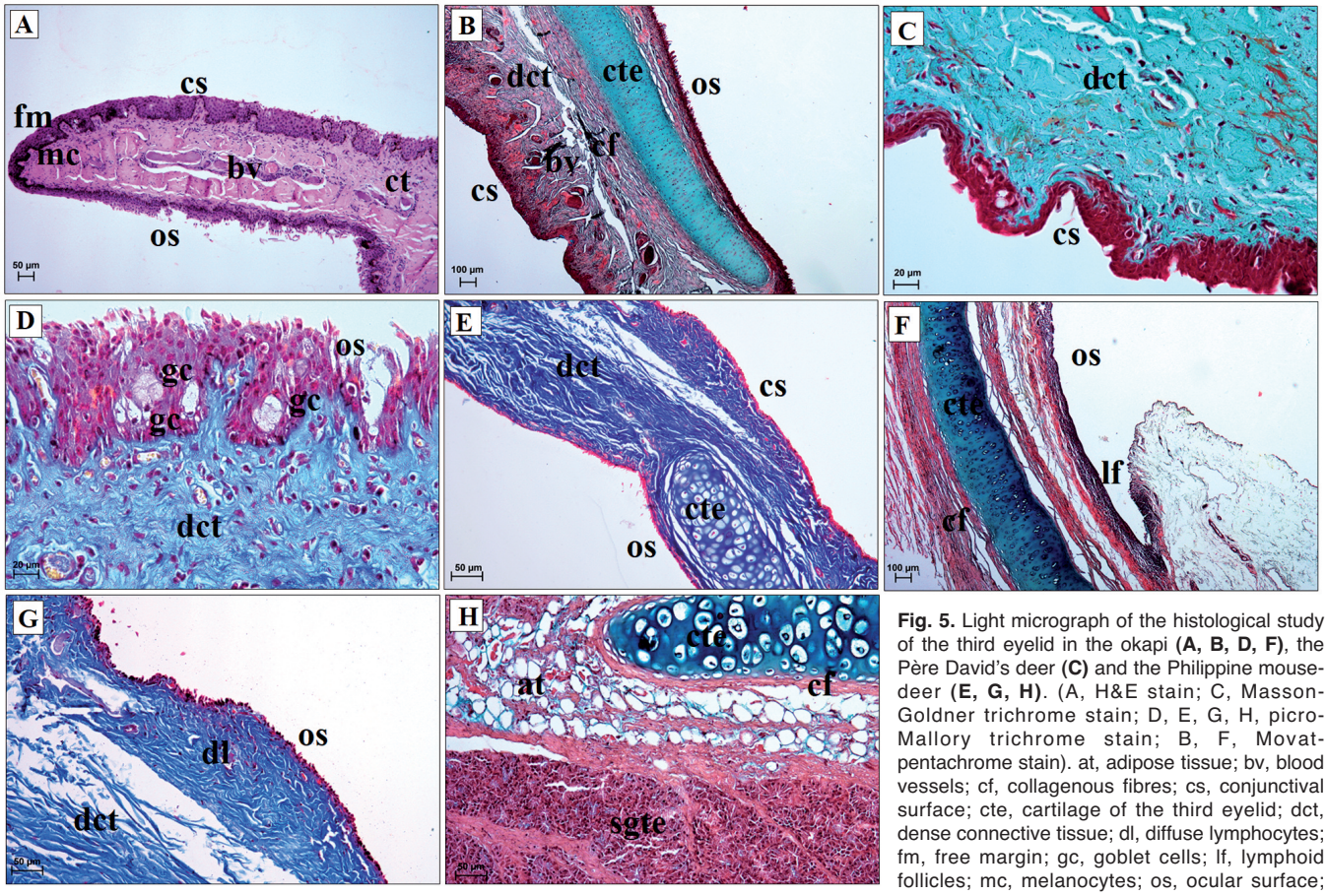

Fig. 5. Light micrograph of the histological study of the third eyelid in the okapi **(A, B, D, F)**, the Père David's deer **(C)** and the Philippine mouse-deer **(E, G, H)**. (A, H&E stain; C, Masson-Goldner trichrome stain; D, E, G, H, picro-Mallory trichrome stain; B, F, Movat-pentachrome stain). at, adipose tissue; bv, blood vessels; cf, collagenous fibres; cs, conjunctival surface; cte, cartilage of the third eyelid; dct, dense connective tissue; dl, diffuse lymphocytes; fm, free margin; gc, goblet cells; lf, lymphoid follicles; mc, melanocytes; os, ocular surface; sgte, superficial gland of the third eyelid. Scale bars: A, E, G, H, 50 µm; B, F, 100 µm; C, D, 20 µm.

examined ruminants was oval and light pink. It was located between the medial rectus and ventral rectus muscles and was partially covered by the ventral oblique muscles. It was uniform and undivided. The largest superficial gland of the third eyelid was present in the okapi, while the smallest one was found in the Philippine mouse-deer (Table 4).

Histological study

The superficial gland of the third eyelid in the okapi and Père David's deer was surrounded by a thick connective tissue capsule (containing collagen and elastic fibers, blood vessels and fibrocytes), which penetrated the gland forming thin septa dividing the gland into large lobes (Fig. 6A,B, Table 5). In the Philippine mouse-deer this gland was covered by a thin connective tissue capsule in comparison to the other two analysed ruminants and the connective tissue septa divided the gland into single small lobes and large lobes contained single adipocytes (Fig. 6C, Table 5).

The superficial gland of the third eyelid in the okapi had a multilobar tubular complex structure (Fig. 6D) and the mucous units formed branched tubular secretory portions (Fig. 6E), while in the Père David's deer (Fig. 6F) and the Philippine mouse-deer, it had a multilobar tubuloacinar structure. In the Père David's deer, the acini were composed of a small lumen composed of tall conical cells with a basophilic cytoplasm, while the tubules with a large lumen were composed of a single layer of cubic cells. The Movat-pentachrome stain showed the presence of numerous mucous acini in this gland (Fig. 6G). In the Philippine mouse-deer, the secretory units contained also a small lumen and were composed of tall conical cells. In all three examined species these tall cells were surrounded by basal myoepithelial cells. Weakly stained mucous acini were present following the Movat-pentachrome stain. Tubules with a large lumen were composed of a single layer of cubic cells.

The superficial gland in the okapi had numerous characteristically short, poorly branched main ducts in

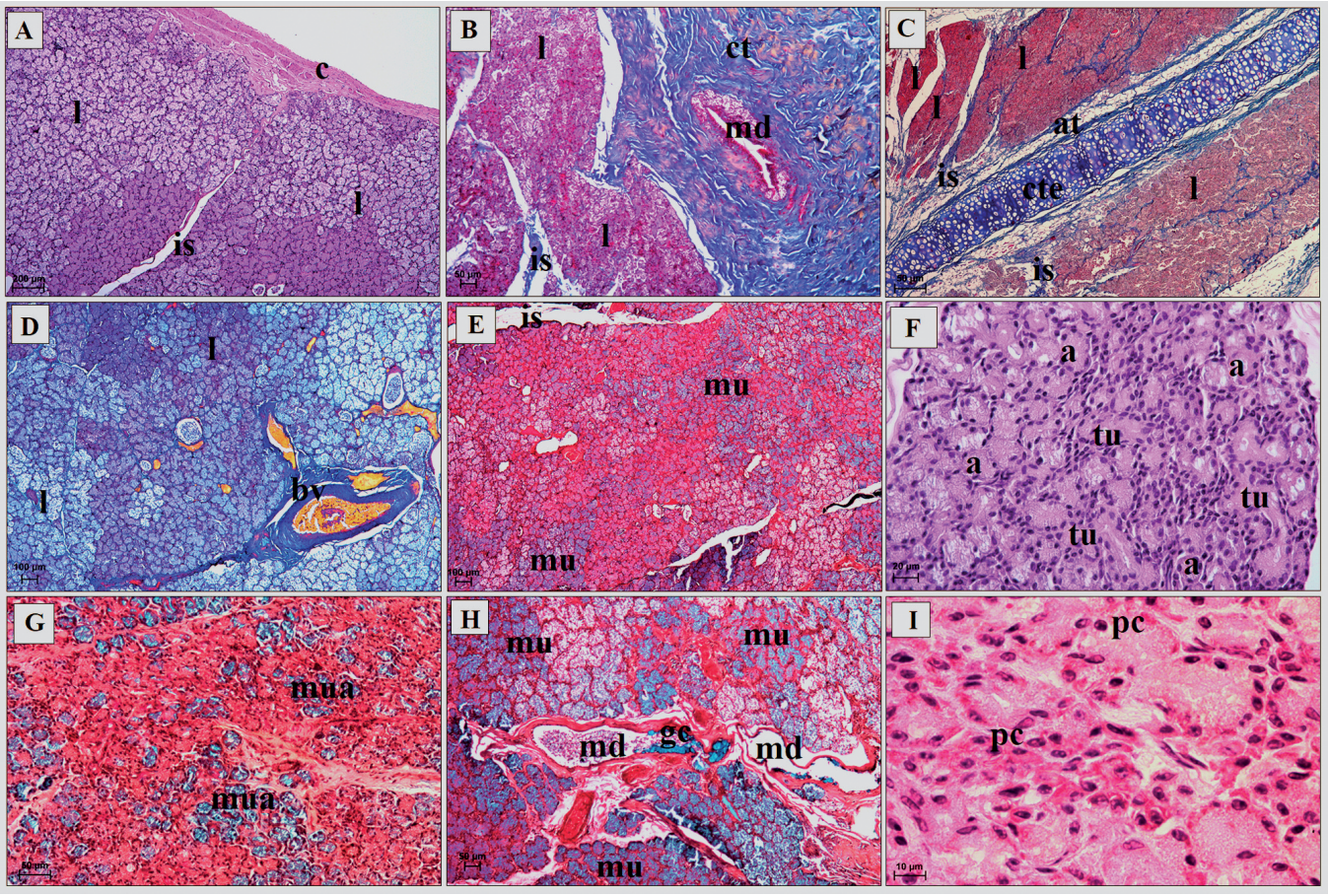

Fig. 6. Light micrograph of the histological study of the superficial gland of the third eyelid in the okapi **(A, D, E, H)**, the Père David's deer **(B, F, G)** and the Philippine mouse-deer **(C, I).** (A, F, H&E stain; B, D, picro-Mallory trichrome stain; E, G, H, Movat-pentachrome stain; C, azan trichrome stain; I, methyl green pyronian Y stain). Scale bars: A, 200 μm; B, C, G, H, 50 μm; D, E, 100 μm; F, 20 μm; I, 10 μm.

the interlobar septa (Fig. 6H). They were composed of a simple columnar epithelium with goblet cells (Fig. 6H). On the other hand, a single unbranched main duct, which consisted of a simple cuboid epithelium with single goblet cells, was present in the Père David's deer. There were no main ducts in the interlobar septa in the studied samples of Philippine mouse-deer.

The MGP Y staining did not reveal plasma cells in the glandular interstitium in the okapi, and showed single plasma cells in the glandular interstitium of the Père

Table 5. Morphometric parameters (*μ*m) of the superficial gland of the third eyelid (SGTE), the deep gland of the third eyelid (DGTE) and lacrimal gland (LG) in the okapi, the Père David's deer and the Philippine mouse-deer.

Examined gland	Species	Capsule thickness (*μ*m)	Interlobar septal thickness (*μ*m)	Serous acini - outer diameter (*μ*m)	Mucous acini -outer diameter (*μ*m)	Tubular diameter (*μ*m)
SGTE	Okapi	373.72±23.9	76.36±21.01	N/A	N/A	60.12±9.2
	Père David's deer	705.47±15.96	141.33±52.8	N/A	39.11±3.1	72.65±3.7
	Philippine mouse-deer	146.51±8.3	95.49±24.9	27.12±1.7	N/A	35.19±1.5
DGTE	Okapi	N/A	N/A	N/A	N/A	N/A
	Père David's deer	163.1±20.9	105.24±53.6	30.57±3.5	41.26±3.8 mucouserous acini	55.41±3.7
	Philippine mouse-deer	523.64±41.8	221.23±19.5	21.26±0.4	N/A	55.42±1.7
LG	Okapi	746.38±145.27	370.11±80.4	N/A	N/A	63.81±12.9
	Père David's deer	383.55±28.2	194.73±53.6	31.37±2.1	30.25±3.3	55.88±11.2
	Philippine mouse-deer	159.86±10.8	85.79±4.7	25.82±2.5	22.78±1.9	43.22±2.3

Values are expressed as mean ± standard deviations. N/A, not analysed.

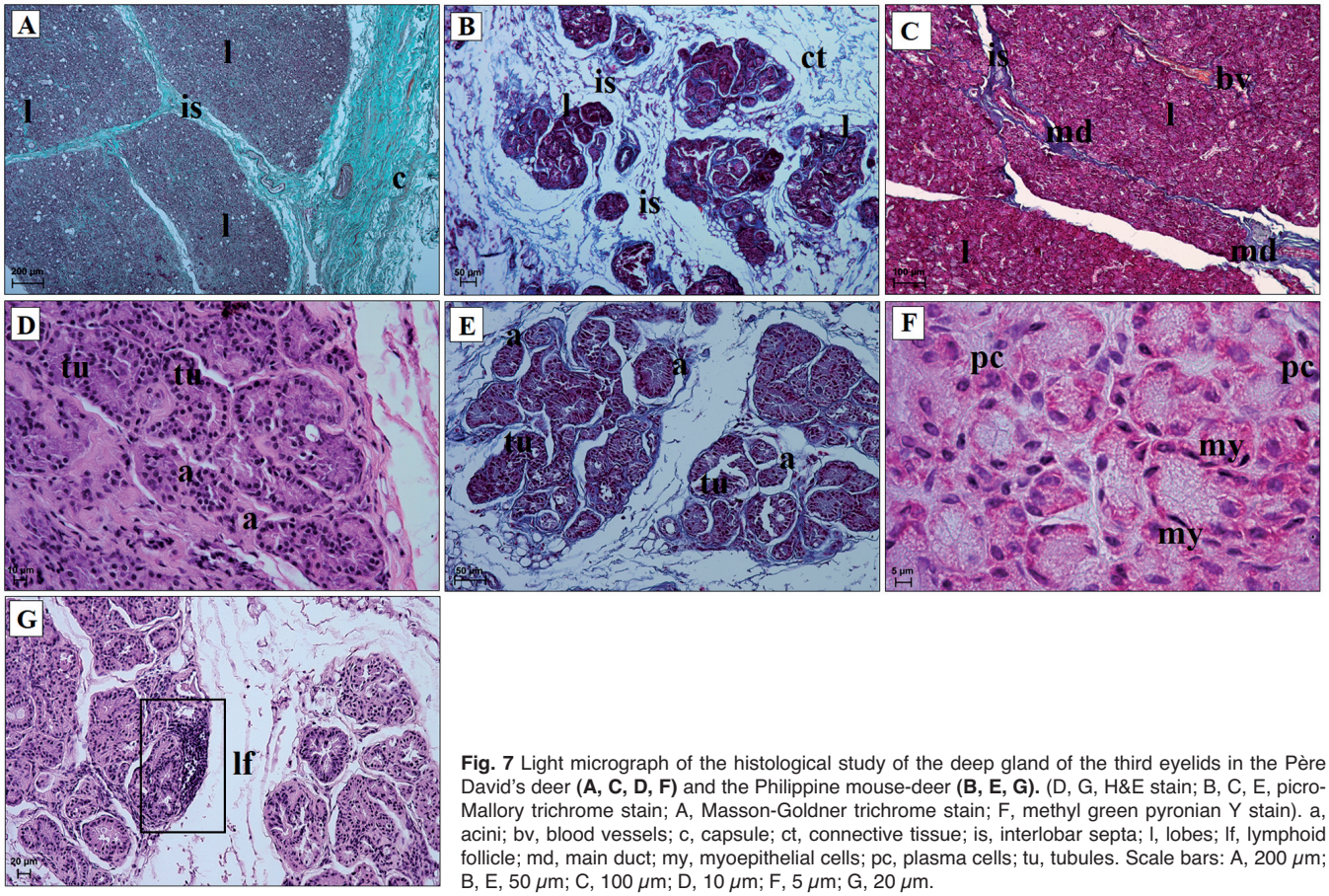

Fig. 7 Light micrograph of the histological study of the deep gland of the third eyelids in the Père David's deer **(A, C, D, F)** and the Philippine mouse-deer **(B, E, G).** (D, G, H&E stain; B, C, E, picro-Mallory trichrome stain; A, Masson-Goldner trichrome stain; F, methyl green pyronian Y stain). a, acini; bv, blood vessels; c, capsule; ct, connective tissue; is, interlobar septa; l, lobes; lf, lymphoid follicle; md, main duct; my, myoepithelial cells; pc, plasma cells; tu, tubules. Scale bars: A, 200 *μ*m; B, E, 50 *μ*m; C, 100 *μ*m; D, 10 *μ*m; F, 5 *μ*m; G, 20 *μ*m.

David's deer with a characteristic nucleus and cytoplasm as well as in the Philippine mouse-deer (Fig. 6I).

The deep gland of the third eyelid

Gross anatomy

The deep gland of the third eyelid in the Père David's deer and the Philippine mouse-deer was located on the ventral rectus muscle in the medioventral part of the periorbita. This gland contacted the superficial gland of the third eyelid and the ventral oblique muscle. There was a single duct on the medial surface of the gland, which opened on the orbital surface of the third eyelid. The deep gland of the third eyelid in the Père David's deer and Philippine mouse-deer was almost round and slightly pink. The Père David's deer had the largest deep gland of the third eyelid (Table 4), while the gland was absent in the okapi.

Histological study

In Père David's deer and the Philippine mouse-deer, the deep gland of the third eyelid was a multilobar tubuloacinar gland covered by a thick connective tissue capsule consisting of collagen and elastic fibers (Fig. 7A,B, Table 5). It contained numerous adipose cells in both species, and these were present in the interlobar septae in the Philippine mouse-deer. The interlobar septae divided the gland into numerous large lobes and single smaller ones in Père David's deer (Fig. 7C). In the Philippine mouse-deer, the septae divided the parenchyma into single small lobes.

In Père David's deer, glandular lobes were formed from dominating serous secretory cells with a small lumen and tall conical cells with a basophilic cytoplasm and a second, less abundant type of mucoserous cells (Fig. 7D). The latter cells formed crescent-shaped patches with mucous tubule cells surrounded by serous cells, creating serous demilunes, confirmed using the Movat-pentachrome stain. In contrast to Père David's deer, the secretory units in the Philippine mouse-deer contained a small lumen composed of tall conical cells with an eosinophilic cytoplasm (Fig. 7E). The Movat-pentachrome stain did not reveal any mucous acini (negative reaction). The tubules in the Père David's deer

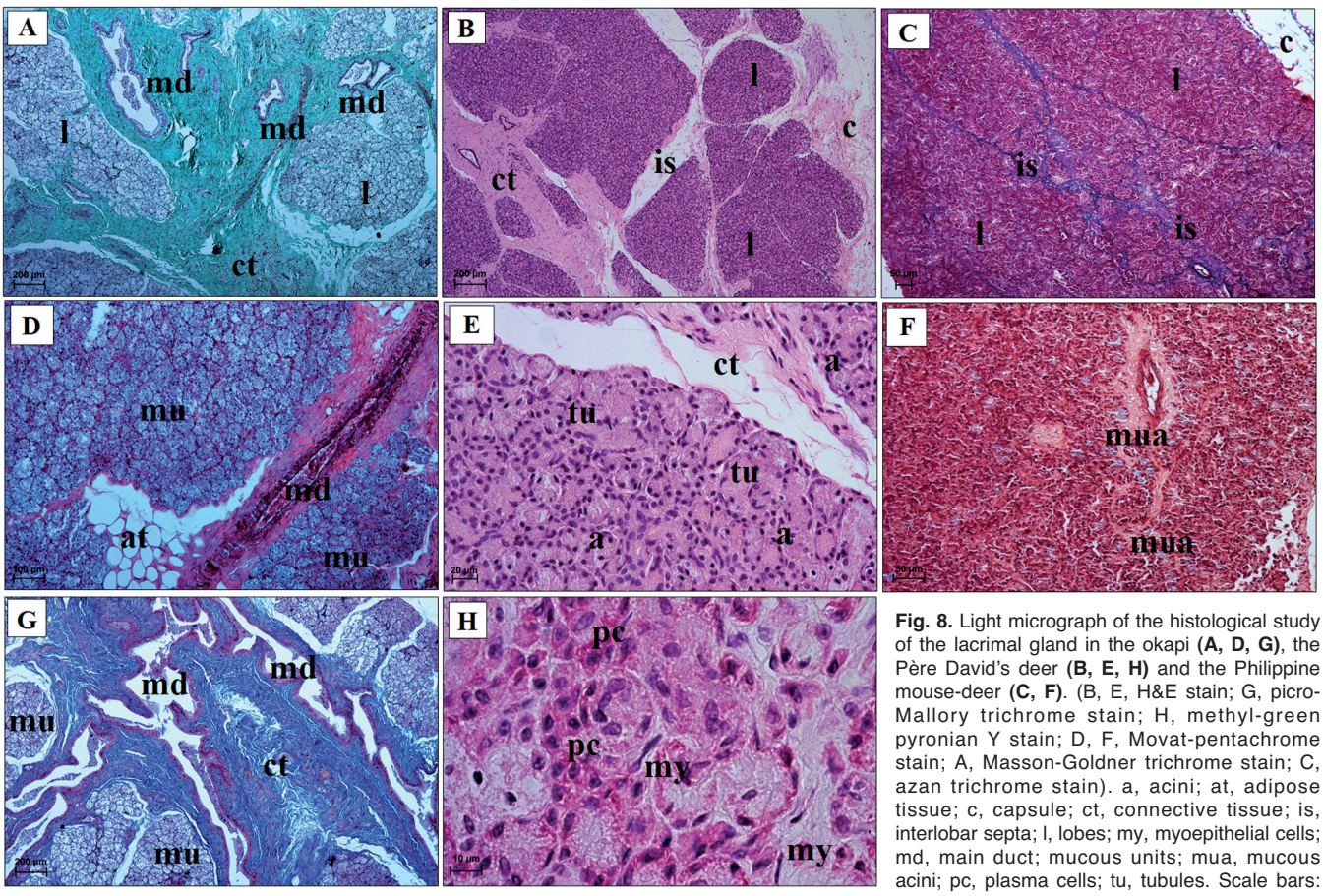

Fig. 8. Light micrograph of the histological study of the lacrimal gland in the okapi **(A, D, G)**, the Père David's deer **(B, E, H)** and the Philippine mouse-deer **(C, F)**. (B, E, H&E stain; G, picro-Mallory trichrome stain; H, methyl-green pyronian Y stain; D, F, Movat-pentachrome stain; A, Masson-Goldner trichrome stain; C, azan trichrome stain). a, acini; at, adipose tissue; c, capsule; ct, connective tissue; is, interlobar septa; l, lobes; my, myoepithelial cells; md, main duct; mucous units; mua, mucous acini; pc, plasma cells; tu, tubules. Scale bars: A, B, E, G, 200 µm; C, F, 50 µm; D, 100 µm; H, 10 µm.

and Philippine mouse-deer had a large lumen and were composed of a single layer of cubic cells.

In the Père David's deer, single and poorly branched main duct was visible in the connective tissue stroma, composed of a simple cuboid epithelium with goblet cells, while in Philippine mouse-deer this duct was absent.

The MGP Y stain showed numerous plasma cells in Père David's deer (Fig. 7F) and numerous clusters of plasma cells in the glandular interstitium of the deep gland and a single lymph follicle in the Philippine mouse-deer (Fig. 7G).

The lacrimal gland

Gross anatomy

All the examined lacrimal glands were light pink. The lacrimal gland in the Père David's deer and Philippine mouse-deer consisted of two parts: the orbital part and the palpebral part. The lacrimal gland (orbital part) was flat and triangular in the Père David's deer and elongated in the okapi and Philippine mouse-deer. The orbital part was located in the dorsolateral angle of the periorbita between the dorsal rectus and lateral rectus muscles. The palpebral part of the gland was located within the superior palpebra. The secretion from the gland entered the conjunctival sac through multiple small excretory ductules, which opened into the upper conjunctival fornix. The lacrimal gland was the longest and widest in the Père David's deer and the smallest in the Philippine mouse-deer (Table 4).

Histological study

The histological examination revealed the presence of a palpebral part of the lacrimal gland in the Père David's deer (Fig. 4A) and the Philippine mouse-deer (Fig. 4B). The lacrimal gland was a branched multilobar tubular complex gland (Fig. 8A) in the okapi, and a multilobar tubuloacinar gland in Père David's deer (Fig. 8B) and the Philippine mouse deer (Fig. 8C). It was covered by a connective tissue capsule, which consisted of collagen fibers, elastic fibres and blood vessels and was surrounded by adipose tissue in the okapi and Père David's deer (Table 5). The connective tissue capsule was the thickest in the okapi (Table 5). The absence of adipose tissue in the connective tissue capsule seemed to be a feature of the Philippine mouse-deer. The capsular connective tissue penetrated into the glandular parenchyma forming septae, which divided the gland into big lobes in the okapi and Philippine mouse-deer (Fig. 8C) and into numerous large and single small lobes in Père David's deer (Fig. 8B). In the okapi, the lobes were composed of mucous units forming tubular secretory portions, which were branched and folded (Fig. 8D). The mucous units were surrounded by myoepithelial cells. In the Père David's deer, the serous

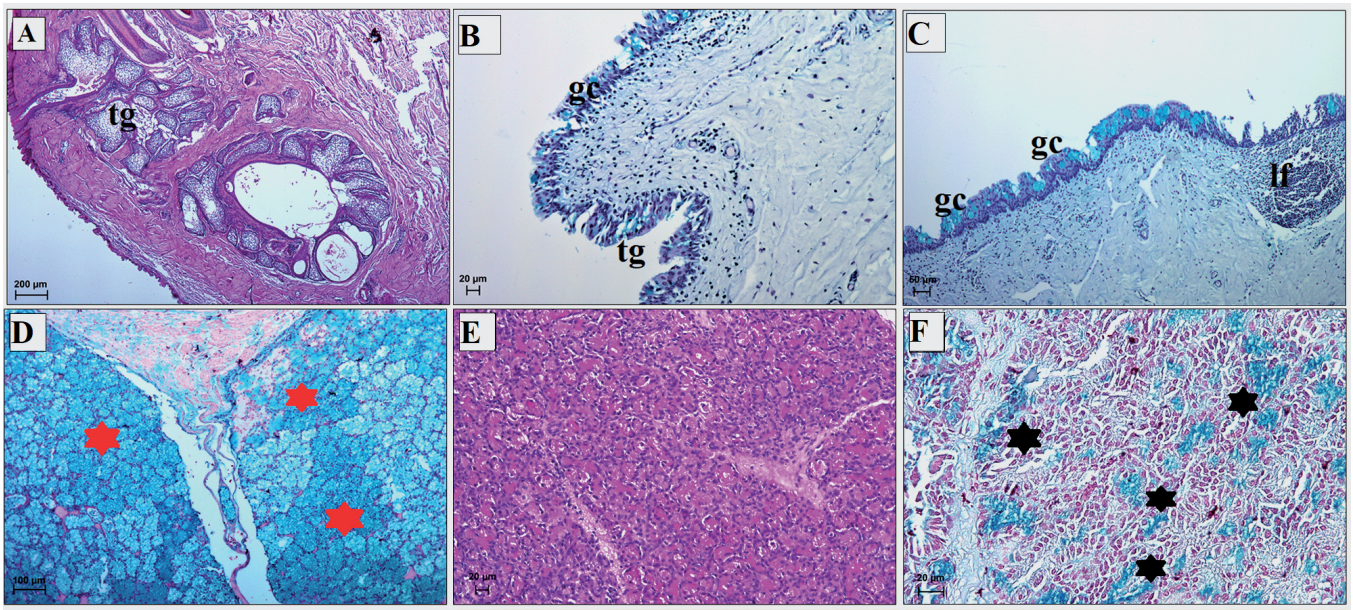

Fig. 9. Light micrograph of the histochemical study of the upper and lower eyelids **(A, B)**, the third eyelids **(C)**, the superficial gland of the third eyelid **(D, E)**, the deep gland of the third eyelid and the lacrimal gland **(E)** in the okapi **(D)**, the Père David's deer **(A, C, E)** and the Philippine mouse-deer **(F)**. (A, E, PAS stain; C, alcian blue pH 2.5 stain; D, F, HDI stain). gc, goblet cells; lf, lymphoid follicle; sg, sebaceous gland; swg, sweat gland; tg, tarsal gland; red asterisk, HDI positive reaction rated as +/++ **(D)** in the superficial gland of third eyelid in the okapi, PAS positive reaction rated as +/++ **(E)** in the lacrimal gland in the Père David's deer, black asterisk - middle (++) HDI positive reaction **(F)** in the superficial gland of third eyelid in the Philippine mouse-deer. Scale bars: A, 200 µm; B, E, F, 20 µm; C, 50 µm; D, 100 µm.

acini containing an irregular lumen were composed of tall conical cells with a basophilic granular cytoplasm (Fig. 8E). The myoepithelial cells were located between the secretory cells and the basal lamina. The Movat-pentachrome stain revealed the presence of single mucous acini. In contrast to Père David's deer, the acini in the Philippine mouse-deer were composed of tall conical cells with an eosinophilic cytoplasm. The Movat-pentachrome staining showed the presence of single weakly stained acini with mucous character (Fig. 8F). In the Père David's deer and Philippine mouse deer, tubules that had a large lumen were composed of a single layer of cubic cells.

Numerous long main ducts in the interlobar connective tissue were a feature of the okapi lacrimal gland (Fig. 8G). The main ducts were composed of a simple columnar epithelium with goblet cells. In the Père David's deer, single and poorly branched main ducts were present in the interlobar septa, while there was no main duct in the Philippine mouse-deer.

In the okapi, no plasma cells were observed in the glandular interstitium. On the other hand, the MGP Y staining showed numerous plasma cells in Père David's deer (Fig. 8H), while the lacrimal gland of the Philippine mouse-deer had single plasma cells located in the interstitium of the gland.

Histochemical study

The histochemical analysis revealed that the tarsal glands and sebaceous glands showed a PAS and Alcian blue pH 2.5 negative reaction, while the sweat glands gave a weakly PAS and Alcian blue pH 2.5 positive reaction (Fig. 9A, Table 6). The goblet cells located in the conjunctival epithelium in both eyelids gave a moderately PAS and Alcian blue pH 2.5 positive reaction (Fig. 9B). In contrast, the goblet cells in the conjunctival and ocular surface of the third eyelid gave a strongly PAS and Alcian blue pH 2.5 positive reaction (Fig. 9C).

The histochemical study indicated that the superficial gland of the third eyelid and lacrimal gland in the okapi secreted a mucous substance (Fig. 9D, Table 6). The goblet cells of the main ducts in the orbital gland in the okapi and Père David's deer were strongly PAS and Alcian blue pH 2.5 stain positive and moderately HDI positive. The superficial gland of the third eyelid, the deep gland of the third eyelid and the lacrimal gland in Père David's deer contained a mucoserous secretion (Fig. 9E, Table 6). In the Philippine mouse-deer, these glands contained a serous secretion (Fig. 9F, Table 6).

Discussion

In the present study, several different features of the macroscopic and microscopic structures of the upper, lower and third eyelids, superficial gland of the third eyelid, deep gland of the third eyelid and lacrimal gland were found in captive ruminants. The changes observed in the three studied species of the Artiodactyla order may be understood within an ecological context and may be associated with different habitat-specific environmental conditions. The okapi or Philippine mouse-deer inhabit damp forest areas, while the Père David's deer is currently found only in captivity. However, it used to inhabit swampy areas. A genetical analysis of the Père David's deer confirmed that it descends from an ancient introgressive hybridization event between parent species that were not closely related (Pitra et al., 2004). Moreover, the biological rhythm may affect the morphology of certain glands, particularly in males that are active during the

Table 6. Histochemical analysis of the upper, lower and third eyelids, superficial gland of the third eyelid, deep gland of the third eyelid and lacrimal gland of the okapi, Père David's deer and Philippine mouse-deer.

Species	Region	PAS	Alcian blue pH 2.5	HDI
Histochemical features of selected organs in the three examined species				
	tarsal glands	-	-	N/A
	sebaceous glands	-	-	N/A
	sweat glands	+	+	N/A
	conjunctival surface of the upper and lower eyelids - goblet cells	++	++	N/A
	conjunctival and ocular surface of the third eyelid - goblet cells	+++	+++	N/A
Okapi	SGTE	+++	++	+/++
	LG	++	+++	+++
	main ducts of LG	+++	+++	+
Père David's deer	SGTE	+++	++/+++	++/+++
	DGTE	++	++	+/++
	LG	+/++	+	+/++
Philippine mouse-deer	SGTE	-/+	-/+	++
	DGTE	+/++	++	-/+
	LG	++	++	+/++

N/A, not analysed.

reproduction season, which is evident in Cervidae (Pitra et al., 2004). The natural habitat of the okapi, endemic to Congo, and the Phillipine mouse-deer, endemic to Indonesia, also significantly impacted the development of certain morphological traits in these species.

The upper and lower eyelids

The upper and lower eyelids in the examined ruminants had a similar anatomical and histological structure other mammals (Dyce et al., 1996; Nickel et al., 2004; Gellat, 2007) although there were some differences in the eyelid structure and the conjunctiva-associated lymphoid tissue (CALT).

The eyelash morphology differed between the studied species. Long and thick eyelashes were present on the upper eyelid of the Père David's deer, which suggests they serve a greater protective role in this species than in the okapi or the Philippine mouse-deer. In the latter two species, the lower eyelid did not contain any eyelashes, while the eyelashes of the upper eyelid were delicate. Hence, the folds of the upper and lower eyelid played a major protective role in those species. Contrary to our findings, Constanstinescu and Moore (1998) reported that eyelashes were present in both eyelids in small ruminants. However, the eyelashes of the upper eyelid were more developed than those of the lower eyelid in the ox (Diesem, 1975), which is similar to the Père David's deer. The eyelashes in the camel were also reported to be well developed (Ibrahim, 1990), which is associated with the habitat that was significantly different to that of the Philippine mouse-deer and the okapi. These species naturally inhabit wetlands, and their mucous membranes are not exposed to conditions causing dry-eyes.

In the orbital zone of the lymphoid region, there was no significant difference in the number of layers of cells between the three examined species. However, a strongly positive PAS and AB pH 2.5 reaction in this area was similar to the findings in the dromedary camel (*Camelus dromedarius*) (Al-Ramadan, 2015). The non-lymphoid region of the orbital zone in the Philippine mouse-deer was similar to those in cattle (Bayraktaroglu and Asti, 2009). In contrast, there were single or double layers of squamous epithelial cells with numerous goblet cells in the okapi and the Père David's deer. In mammals and adult birds, the CALT consists of numerous lymphoid follicles located immediately beneath the conjunctival epithelium of the upper and lower palpebra and numerous disseminated follicles located in the lamina propria of the upper and lower mucous membrane (Fix and Arp, 1991; Khan et al., 2007; Steven et al., 2008). In the current study organized lymphoid follicles and diffuse lymphocytes - CALT were observed in the upper and lower eyelids in the okapi and Père David's deer, but the CALT was mainly located in the lower eyelid rather than the upper eyelids, which differed from cattle (Bayraktaroglu and Asti, 2009). In the Philippine mouse-deer, diffuse lymphocytes were present in both eyelids, which had previously been reported by Al-Ramadan (2015) in the dromedary camel and in Angora goats (Asti et al., 2000). In humans and animals, the ocular immune system protects the eye from different diseases which cause conjunctivitis, e.g. bacterial, viral and toxic infections, allergy and the dry-eye syndrome (Siebelmann et al., 2013; Oria et al., 2014). Although the Philippine mouse-deer has poorly-developed eyelashes, it contains CALT in both eyelids, which suggests that the immune system plays an important protective role against foreign bodies and microorganisms in this species. In the three studied species, our findings of the morphological features of the eyelid glands were consistent with those carried out in other animals, where the tarsal glands in both eyelids formed large yellow columns arranged parallel to each other and perpendicular to the posterior palpebral margin (Fahmy et al., 1971; Hifny et al., 1985; Gilbart et al., 1989; Hoffman et al., 1997; Sullivan et al., 1998; Rehorek et al., 2010; Al-Ramadan, 2015). Numerous sebaceous and sweat glands in the eyelid stroma of both eyelids were comparable to those in other ruminants: cattle, dromedary camels and the brown brocket deer (Ikeda, 1953; Ibrahim et al., 1992; Ajmat et al., 2004; Nickel et al., 2004; Al-Ramadan, 2015). Conversely, according to Diesem (1975), the sebaceous glands in sheep and goats are not associated with hair follicles and the main duct is lined with a stratified squamous epithelium.

The superficial gland of the third eyelid

The superficial gland of the third eyelid was the largest in the okapi and was circular in all the studied ruminants. Its location was similar to that in other Artiodactyla (Fahmy et al., 1971; Dyce et al., 1996; Schlegel et al., 2001; Pinard et al., 2003b; Rehorek et al., 2007; Mohammadpour, 2009; Al-Ramadan and Ali, 2012; Klećkowska-Nawrot et al., 2015a,b). The gland size was directly associated with the size of the animal as the smallest gland was found in the Philippine mouse-deer. We did not study the relationship between body weight and the weight of individual glands. It would also be interesting to compare the gland size depending on the stage of the reproductive cycle in individual species. An oval-shaped superficial gland was reported in roe deer (Capreolus capreolus) (Klećkowska-Nawrot et al., 2013), in the one-humped camel (Mohammadpour, 2009; Al-Ramadan et Ali, 2012) and alpaca (Klećkowska-Nawrot et al., 2015a), while it had a triangular shape in the dromedary camel (Fahmy et al., 1971; Ibrahim, 1990). The shape of this gland in our study was different to the superficial gland in the European bison in which this gland was bipartite with an accessory lobe (Klećkowska-Nawrot et al., 2015b).

In the examined species, the histological analysis revealed that the superficial gland of the third eyelid was similar to the same gland in the alpaca (*Vicugna pacos*), Lori sheep and the European bison (Abbasi et al., 2014;

Klećkowska-Nawrot et al., 2015a, 2015b). The histochemical findings in the present study revealed that the superficial gland in the okapi had a mucous character similar to that in the one-humped camel (Al-Ramadan and Ali, 2012). In the Père David's deer, the glands had a mucoserous secretion similar to that in the European bison, alpaca and roe deer (Klećkowska-Nawrot et al., 2013, 2015a, 2015b) but different from that in Philippine-mouse deer. According to Rehorek et al., (2007), the superficial gland in the Chinese muntjac contains typical pyramidal seromucous cells that react PAS positively. In the fallow deer, these cells showed an M-BPB positive reaction signifying the presence of protein and an ORO positive reaction signifying the presence of lipids.

The third eyelid

Small ruminants and the one-humped camel were reported to have a similar third eyelid shape to both Père David's deer and the Philippine mouse-deer, while an anchor shape of the third eyelid was found in the alpaca and European bison (Schlegel et al., 2001; Al-Ramadan and Ali, 2012; Klećkowska-Nawrot et al., 2015a, 2015b). Schlegel et al. (2001) described the cartilage in small ruminants as a thin rod that extended distally in a slightly curved form in a crescent-like shape.

Similar histological structure findings in the third eyelid were observed in domestic and wild ruminants (Fahmy et al., 1971; Smollich and Michel, 1992; Mohammadpour, 2009; Klećkowska-Nawrot et al., 2015a, 2015b; Salman, 2016). In contrast to our study, the cartilage of the third eyelid in the one-humped camel was composed of elastic tissue (Schlegel et al., 2001; Al-Ramadan and Ali, 2012).

Our study found numerous lymph follicles in the lymphoid region of the ocular surface of the third eyelid in the okapi and Père David's deer. Al-Ramadan and Ali (2012), Banks (1993), Ibrahim (1990), and Hifny and Aly (2006) also reported that the third eyelid contained many lymph follicles and diffuse lymphocytes scattered in the subconjunctiva along the bulbar surface in the one-humped camel, ox, sheep and buffalo. In the Philippine mouse-deer, the bulbar surface of the third eyelid only contained diffuse lymphocytes.

The deep gland of the third eyelid

The deep gland of the third eyelid was present in Père David's deer and the Philippine mouse-deer and it is comparable to other ruminants, such as the European bison, roe deer doe, American bison, cattle, Chinese muntjac (*Muntiacus reevesi)* and fallow deer (*Dama dama*) (Pinard et al., 2003b; Rehorek et al., 2007; Klećkowska-Nawrot et al., 2008; 2015b). However this gland had different shapes in different mammals. It was elongated and tubular with a smooth exterior in the American bison (Pinard et al. 2003b), more triangular in cattle, and oval in the European bison and roe deer doe (Klećkowska-Nawrot et al., 2008; 2015b). In our study, the gland differed in colour between animals. The difference was also observed between the one-humped camel, where it was pinkish or reddish (Fahmy et al., 1971) and the buffalo and the camel where it was brown (Ibrahim, 1990). Diesem (1975) reported that similarly to okapi, this gland was absent in sheep and goats, however the superficial gland of the third eyelid in these species was large.

Compared to the same gland in the Père David's deer and other ruminants, the deep gland in Philippine mouse-deer had a connective tissue capsule, which did not appear as a distinct compact structure but formed thick interlobar septae that divided the parenchyma into single lobes. Interestingly, although the connective tissue capsule was the smallest in the Philippine mouse deer, it was thickest if regarded in relation to the size of the entire organ. Salman (2016) reported that in the local buffalo, this gland consisted of acinar serous and mucous units lined by simple columnar epithelium tissue. According to Klećkowska-Nawrot et al., (2015b), the deep gland in the European bison was similar to that of the Philippine mouse-deer. Hraste et al. (1996) reported that the acini in the deep gland of the red deer doe (Cervus elaphus) and the roe deer doe (Capreolus capreolus) contained highly prismatic glandular cells, which were arranged more densely in the roe deer doe than in the red deer doe. Our study showed some differences between Père David's deer and the Philippine mouse-deer, which were also reported in cattle (Pinard et al., 2003b), the Chinese muntjac (Rehorek et al. 2007), the dromedary camel (Abuelhassan, 1999) or Bubali bubalis (Salman, 2016).

We also noted some differences based on the histochemical analysis, which indicated the presence of cells producing different secretions depending on the species and natural habitat of the animals. This was also reported in the European bison (Klećkowska-Nawrot et al., 2015b), American bison and cattle (Pinard et al., 2003b), Chinese muntjac and fallow deer (Rehorek et al., 2007) and dromedary camel (Abuelhassan, 1999).

The lacrimal gland

Our study revealed the presence of a palpebral part of the lacrimal gland in the Père David's deer and the Philippine mouse-deer, which is also found in humans (Obata, 2006). The lacrimal gland was similar in appearance in the European bison, the alpaca, the roe deer, the Lori sheep and the Philippine buffalo (*Bubalus bubalis*), where it was undivided and distinctly lobulated. In cattle, on the other hand, the lacrimal gland had an accessory lobe (Pinard et al., 2003b; Maala et al., 2007; Abbasi et al., 2014; Klećkowska-Nawrot et al., 2013, 2015a, 2015b). Our study showed that the lacrimal gland was the longest in the Père David's deer and that its length was comparable to that in the European bison and American bison (Pinard et al., 2003b; Klećkowska-Nawrot et al., 2015b).

The lacrimal gland secretion differed in the three studied species and was mucous in okapi, mucoserous in the Père David's deer and serous in the Philippine mouse-deer. The studies by Shadkhast and Bigham (2010) on the Iranian River buffalo, by Abbasi et al. (2014) on Lori sheep and by Kühnel (1968c) on the bovine lacrimal gland showed it had a similar structure to that of the Père David's deer. The lacrimal gland in the European bison and alpaca was similar to that of the Philippine mouse deer (Klećkowska-Nawrot et al., 2015a, 2015b). Ceccareli et al. (1998) and Gargiulo et al., (1999) found that the lacrimal gland in sheep had serous, mucous and seromucous types of cells classified on the basis of the granule ultrastructural features, while the lacrimal gland in camels (*Camelus dromedarius*) was composed of serous and mucous acini (Ibrahim and Abdalla, 2015), similarly to Père David's deer.

Conclusions

The detailed analysis of chosen structures of the accessory organs of the eye in three captive ruminants revealed structural differences, especially in the type of glandular tissue. The diverse secretory content, which was determined based on histochemical analyses, may reflect differences in the natural habitats of these species. Future studies are needed for identification of the potential differences between male and female orbital glands morphology in each species of examined ruminants. Additionally some of the features of the glands microstructure especially in male can be related with the season of the year, including the reproduction activity.

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*Competing Interests. T*he authors declare that they have no financial or personal relationships, which may have inappropriately influenced them in writing this article.

Authors' contributions. JKN - conceived the study, collected the material, carried out the histological and histochemical study, interpreted the results, prepared figures and/or tables, wrote the manuscript; KGH - collected the research material, carried out the dissection of the cadavers, performed the morphometric analysis of the results, prepared figures and/or tables and wrote the manuscript; KB – carried out the dissection of the cadavers and edited the manuscript.

References

Abbasi M., Karimi H. and Gharzi A. (2014). Preliminary anatomical and histological study of lacrimal gland in Lori sheep. J. Vet. Sci. Tech. 5, 1-4.

Abuelhassan A.I.A. (1999). Morphology and morphometry of the eyeball and its appendages of the dromedary camel (*Camelus dromedarius*). A thesis submitted in fulfillment of the requirements for the degree of Master of Veterinary Science (M.V.Sc.).

Ajmat M.T., Chamut S. and Black-Decima P. (2004). A histological study of cutaneous glands in the brown brocket deer. Acta Theriol. 49, 93-102.

Aldana Marcos H.J., Ferrari C.C., Cervino C. and Affani J.M. (2002). Hisology, histochemistry and fine structure of the lacrimal and nictitans gland in the South American armadillo (Chaetophractus villosus) (Xenarthra, Mammalia). Exp. Eye Res. 75, 731-744.

Aldana Marcos H.J. and Affani J.M. (2005). Anatomy, histology, histochemistry and fine structure of the Harderian gland in the South American armadillo *Chaetophractus villosus* (Xenarthra, Mammalia). Anat. Embryol. 209, 409-424.

Alexandre-Pires G., Algueró M.C., Mendes-Jorge L., Trindade H., Correia M. and Esperança Pina J.A. (2008). Immunophenotyping of lymphocyte subsets in the third eyelid tissue in dogs (*Canis familiaris*): morphological, microvascular, and secretory aspects of this ocular adnexa. Micosc. Res. Tech. 71, 521-528.

Al-Ramadan S.Y. (2015). Histological features and MUC1 distribution in the palpebral conjunctiva of the dromedary camel (*Camelus dromedarius*). Assiut Vet. Med. J. 61, 179-186.

Al-Ramadan S.Y. and Ali A.M. (2012). Morphological studies on the third eyelid and its related structures in the one-humped camel (*Camelus dromedarius*). Indian J. Vet. Anat. 5, 71-81.

Alsafy M.A.M. (2010). Comparative morphological studies on the lacrimal apparatus of one humped camel, goat, and donkey. J. Biol. Sci. 10, 224-230.

Asti R.N., Kurtdede N., Altunay H. and Ozen A. (2000). Electron microscopic studies on conjunctiva associated lymphoid tissue (CALT) in Angora goats. Deutsche Tierärztl. Wochen. 107, 196-198.

Banks W.J. (1993). Applied veterinary histology. Lea and Fiebiger. Philadelphia AP.

Bayraktaroglu A.G. and Asti R.N. (2009). Light and electron microscopic studies on conjunctiva associated lymphoid tissue (CALT) in cattle. Rev. Méd. Vét. 160, 252-257.

Bayraktaroglu A.G., Korkmaz D., Asti R.N., Kurdede N. and Altunay H. (2011). Conjunctiva associated lymphoid tissue in the ostrich (Struthio camelus). Kafkas Üniv. Vet. Fakult. Derg. 17, 89-94.

Burck N.C. (1975). Technika histologiczna. Warszawa, PZWL.

Carson F. (1990). Histotechnology a self-instructional text. 1st ed. ASCP. pp 126-127.

Ceccarelli P., Coliolo P., Fagioli O. and Gargiulo A.M. (1998). Sheep lacrimal gland: an ultrastructural and histochemical study. Anat. Histol. Embryol. 24, 438.

Constantinescu G.M. and Moore C.P. (1998). Clinical anatomy of the eyelids for small animal practitioners. Wiener Tierärztl. Monats. 85, 229-232.

Das Shina R. and Calhoun M.L. (1966). A gross, histologic and histochemical study of the lacrimal apparatus of the sheep and goats. Am. J. Vet. Res. 27, 1633-1640.

Davidson H.J. and Kuonen V.J. (2004). The tear film and ocular mucins. Vet. Ophthalmol. 7, 71-77.

Diesem D.V.M. (1975). Sense organs and common integuments. In: Sisson and Grossman's. The anatomy of the domestic animals. Revised by R. Getty. W.G.

Ding C., Parsa L., Nadoskar P., Zhao P, Wu K. and Wang Y. (2010). Duct system of the rabbit lacrimal gland: structural characteristics and role in lacrimal secretion. IOVS 51, 2960-2967.

Djeridane Y. (1992). The Harderian gland of desert rodents: a histological and ultrastructural study. J. Anat. 180, 465-480.

Dyce K.M., Sack W.O. and Wensing C.J.G. (1996). Textbook of veterinary anatomy. W.B. Saunders Company. Philadelphia London New York St. Louis Sydney Toronto. pp 333-335.

El-Fadaly A.B., El-Shaarawy E.A.A., Rizk A.A., Nasralla M.M. and Shuaib D.M.A. (2014). Age-related alterations in the lacrimal gland of adult albino rat: A light and electron microscopic study. Ann. Anat. 196, 336-351.

Fahmy M.F.A., Arnautovic I. and Abdalla O. (1971). The morphology of the tarsal glands and the glands of the third eyelid in the one-humped camel (*Camelus dromedarius*). Acta Anat. 78, 40-46.

Fix A.S. and Arp L.H. (1991). Morphologic characterization of conjunctiva-associated lymphoid tissue in chickens. Am. J. Vet. Res. 52, 1852-1859.

Gargiulo A.M., Coliolo P., Ceccarell I.P. and Pedini V. (1999). Ultrastructural study of sheep lacrimal gland. Vet. Res. 30, 345-351.

Gasser K., Fuchs-Baumgartinger A., Tichy A. and Nell B. (2011). Investigations on the conjunctival goblet cells and on the characteristics of glands associated with the eye in the guinea pig. Vet. Ophthalmol. 14, 26-40.

Gellat K.N. (2007). Textbook of veterinary ophthalmology. Lea & Febiger. Philadelphia.

Gilbart J.P., Rossi R.S. and Heyda K.G. (1989). Tear film and ocular surface changes after closure of the meibomian gland orifices in the rabbit. Ophthalmology 96, 1180-1186.

Girgiri I.A. and Kumar P. (2018). Histological and histochemical studies on the lacrimal gland of buffaloes (*Bubalus bubalis*). SJAVS 5, 283-289.

Hifny A., Hassan A.H.S., Selim A.A.A. and Moustafa M.N. (1985). Histological and histochemical studies on the eyelids of the buffaloes in Egypt. Assiut Vet. Med. J. 14, 28-34.

Hifny A. and Aly Kh.A. (2006). Morphological features of the third eyelid in camel. Proceedings of the International Scientific Conference On Camels. Part 3, 1462-1468.

Hoffman D.M., Miller K.V., Marchinton R.L. and Osborn D.A. (1997). Ultrastructure of hairs associated with the skin glands of the white-tailed deer (*Odocoileus virginianus*). Georgia J. Sci. 55, 209-214.

Hraste A., Zobundzija M., Jakovac M., Mihelic D. and Brestovec V.J. (1996). Histological and histoenzymatic properties of glandulae profundae plicae semilunar conjunctivae in a red deer doe (*Cervus elaphus* L.) and a roe deer doe (*Capreolus capreolus* L.). Zeitschrift Jagdwissen. 42, 157-161.

Ibrahim M.T. (1990). Surgical anatomical studies on appendages of the eye in camel, buffalo and donkey. Thesis Submitted for Ph. D. of Anatomy. Department of Anatomy and Histology. Assiut University Egypt.

Ibrahim Z.H. and Abdalla A.B. (2015). Histochemical analysis of glycoconjugates in the lacrimal gland of the Camel (*Camelus dromedarius*). J. Agricul. Vet. Sci. Qassim. Univer. 8, 85-94.

Ibrahim I.A., Kelany A.M. and Taha M. (1992). Comparative anatomical and histological studies on the meibomian (tarsal) glands in rabbits, cats, goats, sheep and cattle. Assiut Vet. Med. J. 28, 81-92.

Ikeda M. (1953). Über die Ciliardrüsen der Säugetiere. Okajimas Folia Anat. Japan 25, 163-168.

Kageyama M., Nakatsuka K., Yamaguchi T., Owen R. L. and Shimada T. (2006). Ocular defense mechanisms with special reference to the demonstration and functional morphology of the conjunctiva-associated lymphoid tissue in Japanese monkeys. Archiv. Histol. Cytol. 69, 311-322.

Khan M.Z.I., Jahan M.R., Islam M.N., Haque Z., Islam M.R. and Kon Y. (2007). Immunoglobulin (Ig)-containing plasma cells in the Harderian gland in broiler and native chickens of Bangladesh. Tissue Cell 39, 141-149.

Klećkowska-Nawrot J. and Dzięgiel P. (2008). Morphology of the lacrimal gland in pig fetuses. Anat. Histol. Embryol. 37, 74-77.

Klećkowska-Nawrot J., Marycz K., Kujawa K., Dzięgiel P., Brudnicki W. and Pospieszny N. (2008). Morphology of the deep gland of the third eyelid in roe-deer (Capreolus capreolus). XIII Kongres Polskiego Towarzystwa Nauk Weterynaryjnych. Olsztyn, 18-20 September.

Klećkowska-Nawrot J., Marycz K., Czogała J., Kujawa K., Janeczek M., Chrószcz A. and Brudnicki W. (2013). Morphology of the lacrimal gland and superficial gland of the third eyelid of roe deer (*Capreolus capreolus* L.). Pak. Vet. J. 33, 139-144.

Klećkowska-Nawrot J., Nowaczyk R., Goździewska-Harłajczuk K., Krasucki K. and Janeczek M. (2015a). Histological, histochemical and fine structure studies of the lacrimal gland and superficial gland of the third eyelid and significance on the proper function of the eyeball in alpaca (*Vicugna pacos*). Folia Morphol. 74, 195-205.

Klećkowska-Nawrot J., Nowaczyk R., Goździewska-Harłajczuk K., Szara T. and Olbrych K. (2015b). Histology, histochemistry and fine structure of the Harderian gland, lacrimal gland and superficial gland of the third eyelid of the European bison (*Bison bonasus bonasus*). Zoologia. 32, 380-394.

Klećkowska-Nawrot J., Goździewska-Harłajczuk K., Barszcz K., Janeczek M. (2016). Functional morphology of the upper and lower eyelids, third eyelid, lacrimal gland and superficial gland of the third eyelid in the red kangaroo (*Macropus rufus*). Folia Biol. 64, 163-181.

Knop N. and Knop E. (2000). Conjunctiva-associated lymphoid tissue in the human eye. IOVS 41, 1270-1279.

Knop E. and Knop N. (2005). The role of eye-associated lymphoid tissue in corneal immune protection. J. Anat. 206, 271-285.

Kurnick N.B. (1955). Pyronin Y in the methyl-green-pyronin histological stain. Stain. Technic. 30, 213-230.

Kühnel W. (1968a). Vergleichende histologische, histochemische und electronen-mikroskopische Untersuchungen an Tränendrüsen. V. Rind. Zeitsch. Zellforsch. Mikroskop. Anat. 87, 504-525.

Kühnel W. (1968b). Vergleichende histologische, histochemische und electronen-mikroskopische Untersuchungen an Tränendrüsen. II. Ziege. Zeitsch. Zellforsch. Mikroskop. Anat. 86, 430-443.

Kühnel W. (1968c). Vergleichende histologische, histochemische und electronen-mikroskopische Untersuchungen an Tränendrüsen. III. Schaf. Zeitsch. Zellforsch. Mikroskop. Anat. 87, 31-45.

Lavach J.D. (1990). Large animal ophthalmology. The C.V. Mosby Company. St. Louis, Baltimore, Philadelphia, Toronto. pp 67-69.

Lee L.G. (1992). Histopathologic methods and color atlas of special stains and tissue artifacts. Johnson Printers, Downers Grove, IL. pp 151-152.

Maala C.P., Cartagena R.A. and De Ocampo G.D. (2007). Macroscopic histological and histochemical characterization of the lacrimal gland of the Philippine water buffalo (*Bubalus bubalus*). Philippine J. Vet. Med. 44, 69-75.

Mallory F.B. (1938). Pathological technique. Philadelphia, WB Saunders Company. pp 72, 76-88.

McKenzie R.W., Jumblatt J.E. and Jumblatt M.M. (2000). Quantification of MUC2 and MUC5AC transcripts in human conjunctiva. IOVS 41, 703-708.

Mohammadpour A.A. (2008). Anatomical characteristics of dorsal lacrimal gland in one humped camel (*Camelus dromedarius*). J. Biol. Sci. 8, 1104-1106.

Mohammadpour A.A. (2009). Morphological and histological study of superior lacrimal gland of the third eyelid in camel (*Camelus dromedarius*). Iranian J. Vet. Res. 10, 334-338.

Movat H.Z. (1955). Demonstration of all connective tissue elements in a single section. Arc. Pathol. 60, 289.

Munakata H., Isemura M. and Yosizawa Z. (1985). An application of the high-iron diamine staining for detection of sulfated glycoproteins (glycopeptides) in electrophoresis on cellulose acetate membrane. Tohoku J. Exp. Med. 145, 251-257.

Nasrin M.K., Khan M.Z.I., Siddiqi M.N.H. and Masum M.A. (2013). Mobilization of immunoglobulin (Ig)-containing plasma cells in Harderian gland, cecal tonsil and trachea of broilers vaccinated with Newcastle disease vaccine. Tissue Cell 45, 191-197.

Nickel R., Schummer A. and Seiferle E. (2004). Lehrbuch der Anatomie der Haustiere. Band IV. Parey Verlag, Stuttgart. pp 432-437.

Nomina Anatomica Veterinaria. (2017). Sixth edition (revised version). Published by the Editoral Committee. Hanover (Germany), Ghent (Belgium), Columbia, MO (U.S.A.), Rio de Janeiro (Brazil).

Obata H. (2006). Anatomy and histopathology of the human lacrimal gland. Cornea 25, S82-S89.

Oria A.P., Gomes Junior D.C., Arraes E.A., Estrela-Lima A., Pinna M.H., Meneses I.D.S. and Martines Filho E.F. (2014). Tear production, intraocular pressure and conjunctival microbiota, cytology and histology of New Zealand rabbits (*Oryctolagus cuniculus*). Pesquisa Vet. Brasileira. 34, 1024-1028.

Payne A.P. (1994). The Harderian gland: A tercentennial review. J. Anat. 185, 1-49.

Pinard C.L., Weiss M.L., Brightman A.H., Fenwick B.W. and Davidson H.J. (2003a). Evaluation of lysozyme and lactoferrin in lacrimal and other ocular glands of bison and cattle and in tears of bison. Am. J. Vet. Res. 64, 104-108.

Pinard C.L., Weiss M.L., Brightman A.H., Fenwick B.W. and Davidson H.J. (2003b). Normal anatomical and histochemical characteristics of the lacrimal glands in the American bison and cattle. Anat. Histol. Embryol. 32, 257-262.

Pitra C., Fickel J., Mejiaard E. and Grove P.C. (2004). Evolution and phylogeny of Old world deer. Mol. Phylogenet. Evol. 33, 880-895.

Rehorek S.J., Hillenius W.J., Sanjur J. and Chapman N.G. (2007). One gland, two lobes: organogenesis of the Harderian and "nictitans" gland of the Chinese muntjac (*Muntiacus reevesi*) and fallow deer (*Dama dama*). Ann. Anat. 189, 434-446.

Rehorek S.J., Hillenius W.J., Leigh C. and Firth B.T. (2010). Is it or isn't it? A reexamination of the anterior orbital glands of the fat-tailed dunnart *Sminthopsis crassicaudata* (Dasyuridae: Marsupiala) and a revolution of definitions for the Harderian gland. Anat. Rec. 293, 1449-1454.

Sakai T. (1981). The mammalian Harderian gland: morphology, biochemistry, function and phylogeny. Arch. Histol. Cytol. 44, 299-333.

Sakai T. (1989). Major ocular glands (Harderian gland and lacrimal gland) of the musk shrew (*Suncus murinus*) with a review on the comparative anatomy and histology of the mammalian lacrimal glands. J. Morphol. 201, 39-57.

Sakai T. (1992). Comparative anatomy of the mammalian Harderian glands. In Harderian glands: Porphyrin metabolism, behavioral and endocrine effects. Webb S.M., Hoffman R.A., Puig-Domingo M.L. and Reiter R.J. (eds). Springer. Berlin. pp 7-23.

Sakai T. and van Lennep E.W. (1984). The Harderian gland in Australian marsupials. J. Mammal. 65, 159-162.

Salman H.A. (2016). Morphological and Histochemical: Study of Harderian gland in Local Buffalo (*Bubalus bubalus*). Int. J. Sci. Tech. 11, 89-92.

Schechter J.E., Warren D.W. and Mircheff A.K. (2010). A lacrimal gland is a lacrimal gland, but rodent's and rabbit's are not human. Ocular Surf. J. 8, 111-134.

Schlegel T., Brehm H. and Amselgruber W.M. (2001). The cartilage of the third eyelid: a comparative macroscopical and histological study in domestic animals. Ann. Anat. 183, 165-169.

Schlegel T., Brehm H. and Amselgruber W.M. (2003). IgA and secretory component (SC) in the third eyelid of the domestic animals: a comparative study. Vet. Ophthalmol. 6, 157-161.

Shadkhast M. and Bigham A.S. (2010). A histo-anatomical study of dorsal lacrimal gland in Iranian river buffalo. Vet Scan. 5, 1-5.

Sheehan D.C. and Hrapchak B.B. (1980). Theory and practice histotechnology. 2nd ed. CV Mosby, St. Louis (MO). pp 52, 164-167.

Siebelmann S., Gehlsen U., Huttmann G., Knop N., Bolke T., Gebert A., Stern M.E., Niederkom J.Y. and Steven P. (2013). Development, alteration and real time dynamics of conjunctiva-associated lymphoid tissue. PLoS One 8, e82355.

Smollich A. and Michel G. (1992). Makroskopische Anatomie der Haustiere. 2 Auflage. Gustav Fischer, Stuttgart Jena. pp 473-476.

Smythe R.H. (1958). Veterinary ophthalmology. Second Edition. Bailliere Tindall and Cox. London.

Spicer S.C. and Henson J.G. (1967). Methods for localizing mucosubstances in epithelial and connective tissue. In: Series on Methods and Achievements in Experimental Pathology. Vol. 2. Bajusz E. and Jamin F. (eds). S Karger Press. Basal. pp 78-112.

Steven P., Rupp J., Hüttmann G., Koop N., Lensing C., Laqua H. and Gebert A. (2008). Experimental induction and three-dimensional two-photon imaging of conjunctiva-associated lymphoid tissue. IOVS 49, 1512-1517.

Stevens A. and Lowe J. (1994). Histology. Gower Medical Publishing. London. pp 205.

Sullivan D.A., Dartt D.A. and Meneray M.A. (1998). Lacrimal gland, tear film and dry eye syndromes 2. Plenum Press, New York. pp 345-348.

Thiessen D.D. and Kittrell E.M.W. (1980). The Harderian gland and thermoregulation in the gerbil (*Meriones unguiculatus*). Physiol. Behav. 24, 417-424.

van Ginkel F.W., Gulley S.L., Lammers A., Hoerr F.J., Gurjar R. and Toro H. (2012). Conjunctiva-associated lymphoid tissue in avian mucosal immunity. Dev. Comp. Immunol. 36, 289-297.

Voigt S., Fuchs-Baumagartinger A., Egerbacher M., Tichy A. and Nell B. (2012). Investigations on the conjunctival goblet cells and the characteristics of the glands associated with the eye in chinchillas (*Chinchilla laniger*). Vet. Ophthalmol. 15, 333-344.

Yasui T., Tsukise A., Nara T., Kuwahara Y. and Meyer W. (2006). Morphological, histochemical and immunohistochemical characterization of secretory productions of the ciliary gland of the porcine eyelid. Eur. J. Histochem. 50, 99-108.

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