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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*)*

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A study concerning the upper (UE) and lower (LE) eyelids, lacrimal gland (LG), superficial gland of the third eyelid (SGTE) and third eyelid (TE) was conducted on 4 sexually mature red kangaroos (2 males and 2 females). Gross anatomical, histological, histometrical, histochemical and ultrastructural (TEM) components were compared. Tissue sections were stained with haematoxylin and eosin, azan-trichrome, van Gieson, Masson-Goldner trichrome, methyl green-pyronin Y, periodic acid-Schiff, alcian blue pH 2.5, aldehyde fuchsin and Hale's dialysed iron. The location of the LG, SGTE, TE, UE and LE was similar to that of other mammals. Organized lymphoid follicles were also found in the LE. The TE resembled the letter T and was composed of cartilage (hyaline tissue). The LG was relatively larger than the SGTE. The LG and SGTE were multilobar tubuloacinar glands. The LG had more plasma cells than the SGTE. The SGTE and LG secretions were mucoserous in composition. The TEM study showed that the secretory cells of the LG and SGTE have a similar ultrastructural appearance. Two types of secretory vesicles were detected in the cytoplasm in acini and one type of secretory vesicle was found in the tubules of these glands.

Key words: Metatheria, Marsupialia, red kangaroo, accessory organs of the eye, morphology.

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The upper and lower eyelids (UE, LE), lacrimal gland (LG), superficial gland of the third eyelid (SGTE) and third eyelid (TE) constitute the accessory organs of the eye (NOMINA ANATOMICA VETERINARIA 2012, NAV).

The UE and LE are dermomuscular folds that surround the palpebral fissure. In animals, the eyelids protect the eyeball and support the maintenance of a moist surface (YASUI *et al.* 2006). The eyelids consist of the three layers: skin with short hairs and a few prominent tactile hairs, a middle musculofibrous layer with tarsus and eyelid glands (tarsal glands, ciliary and sebaceous glands) as well as a mucous membrane, known as the palpebral conjunctiva (CONSTANTINESCU & MOORE 1998; GOLLER & WEYRAUCH 1993; WEYRAUCH 1984; YASUI *et al.* 2006). In the palpebral conjunctiva, there are also accessory lacrimal glands and goblet cells. These glands and goblet cells secrete components of the tear film (BLOOM & FAWCETT 1962; GASSER *et al.* 2011; GOLLER & WEYRAUCH 1993; VAUGHAN & ASBURY 1980;VOIGT *et al.* 2012; YASUI *et al.* 2006). Together with the orbital glands and the conjunctiva-associated lymphoid tissue (CALT) they moisturize the eye and supply the cornea and conjunctival sacs with nutrients and

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antibodies to maintain their health (JOCHEMS & PHILIPS 2015; KLEĆKOWSKA-NAWROT *et al*. 2016).

The morphology of LG has been described in a variety mammals (ABBASI *et al.* 2014; DING *et al.* 2010; DRAPER *et al.* 1998; KLEĆKOWSKA-NAWROT & DZIĘGIEL 2008; KLEĆKOWSKA-NAWROT *et al*. 2013; KLEĆKOWSKA-NAWROT *et al.* 2015a, 2015b; MARTIN *et al.* 1988; OBATA 2006; PINARD *et al.* 2003; REHOREK *et al*. 2007; RIOS *et al.* 2005; SCHECHTER *et al*. 2010). Among these species, there was variability in the number of plasma cells in the gland interstitium (ABBASI *et al.* 2014; GARGIULO *et al*. 1999; MARTIN *et al.* 1988). The LGs produce an aqueous layer of the precorneal tear film composed of three layers. The outermost lipid layer is produced by the tarsal glands, sebaceous glands and ciliary glands. The second middle aqueous layer is produced by the LG and accessory lacrimal glands. The third innermost mucus layer is produced by SGTE and goblet cells of the conjunctiva and crypts of Henle (DAVIDSON & KUONEN 2004; EL-FADALY *et al.* 2014; KLEĆKOWSKA-NAWROT *et al.* 2013). The LG plays an important role in protecting the cornea and the conjunctiva from drying up and also nourishes and lubricates the eye. It also contributes to metabolite exchange, protects the cornea surface from damage caused by foreign bodies and has bactericidal and bacteriostatic properties (ABBASI *et al*. 2014; DAVIDSON & KUONEN 2004; MOHAMMADPOUR 2008; SCHECHTER *et al.* 2010). The LG has an important function in the defense system (B cells and T cells of the immune system as well as plasma cells) of the eyeball and form the lacrimal gland-associated lymphoid tissue (LGALT), (KNOP & KNOP 2000, 2001, 2002, 2003, 2005; PAULSEN *et al.* 2002; KLEĆKOWSKA-NAWROT *et al*. 2016) forming a part of the head-associated lymphoid tissue (HALT) (NASRIN *et al*. 2013; VAN GINKEL *et al.* 2012).

The SGTE, also called the nictitans gland (NAV 2012), has been described in Artiodactyla, Carnivora, Insectiovora, Lagomorpha, Marsupialia, Perissodactyla and Primates (ALDANA MARCOS *et al.* 2002). The SGTE secretion is part of the third mucous layer of 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 (DAVIDSON & KUONEN 2004; MCKENZIE *et al.* 2000).

The TE is also described as a semilunar fold of the conjunctiva (*plica semilunaris conjuncitve*) (NAV 2012) or nictitating membrane. It is found in some elasmobranch fish, amphibians, reptiles, birds and mammals (except humans) and in some primates (lemurs and the lorisoid primates). The role of the third eyelid is mechanical protection of the cornea. It is also responsible for local immunological protection by substances which are present in lymph nodules and are distributed on the corneal surface by tear film distribution (LAVACH 1990; SCHLEGEL *et al.* 2003).

Morphological studies of the UE, LE, LG, SGTE and TE in Marsupialia were carried out in the koala (*Phascolarctos cinereus*) and focused on the macromorphology of the accessory organs of the eye (KEMPSTER *et al.* 2002). Another examination of anterior orbital glands was performed in fat-tailed dunnart (*Sminthopsis crassicaudata*) (REHOREK *et al.* 2010b). Diagnostic ophthalmic tests have been described in the red kangaroo (*Macropus rufus*) and western gray kangaroo (*Macropus fuliginosus*), which aid veterinary ophthalmologists and zoo veterinarians to diagnose ocular diseases (LABELLE *et al.* 2010; TAKLE *et al.* 2010).

The red kangaroo is a common zoo animal. Therefore, zoo veterinarians need to advance their knowledge of diseases occurring in this species and the available treatment options. Ocular diseases significantly impact the condition of the animals and their breeding. It is worth noting that there is no available data on the morphology and morphometry of the accessory organs of the eye of the red kangaroo. The aim of this study was to describe the location as well as the macroscopic and microscopic features of the LE, UP, TE, LG and SGTE of the red kangaroo. The findings will broaden our knowledge of comparative anatomy of wild mammals.

Material and Methods

Animals

The study was conducted on 2 male and 2 female red kangaroos (*Macropus rufus*). The animals were obtained from a private collection of the Department of Morphological Sciences, Faculty of Veterinary Medicine of the Warsaw University of Life Sciences (GOŹDZIEWSKA-HARŁAJCZUK *et al.* 2016). The animals were not killed for the purpose of this study. According to Polish law, studies on tissues obtained *post-mortem* do not require the approval of the Ethics Committee, (PARLIAMENT OF THE REPUBLIC OF POLAND 2012) (Act of Animal Protection passed on August 21, 1997 by the Parliament of the Republic of Poland; No. 111/ 724). Immediately after natural death of the animals, each eyeball with glands from each male and female were collected (n=32 organs), then the measurement of eyelids and glands were performed. Each UE and LE (n = 16; 8 from females and 8 from males) and 8 glands: 4 LG (n = 4; 2 from females and 2 from males) and 4 SGTE (n = 4; 2 from females and 2 from males) were fixed in 4% buffered formaldehyde (for light microscopy). Eight remaining glands four LG (n = 4; 2 from a female and 2 from

a male) and four remaining SGTE (n = 4; 2 from a female and 2 from a male) were fixed in 2.5% glutaraldehyde dissolved in 0.1 M phosphate buffer pH 7.4 for ultrastructural studies (electron microscopy).

Macroscopic study

The eyelids and glands were first examined with the naked eye. They were then studied under the stereoscopic Zeiss Stemi 2000-C microscope (Carl Zeiss, Jena, Germany). A 0.5-4% solution of acetic acid and 70% ethyl alcohol were used for better visualization of the LG and SGTE structures. Typical topographic methods such as holotopy and syntopy were used to describe the morphology of the UE, LE, LG, SGTE and TE. The measurements of the organs were conducted in all collected eyelids and glands using an electronic slide caliper with an accuracy of 0.1 mm. The values of 6 measurements (length, width, thickness) of all collected eyelids and glands were analysed and expressed as mean ± standard deviation (SD) (Table 1). The terminology used in the manuscript is in accordance with the prevailing veterinary nomenclature (Anatomical Veterinary Nomenclature – latin, polish, english 2002; NOMINA ANATOMICA VETERINARIA 2012).

Light microscopy examination

Histological findings

The UE and LE eyelids (n = 16; 8 from females and 8 from males) and glands (n = 8; 4 from females and 4 from males) were directly fixed in 4% buffered formaldehyde for 72 hours and washed in running water for 24 hours. The material was then processed in a vacuum tissue processor – ETP (RVG3, INTELSINT, Italy), embedded in paraffin and cut using a Slide 2003 (Pfm A.g., Germany) sliding microtome into 3-4 µm sections. The samples were then stained with H&E, Azan trichrome, van Gieson and Masson-Goldner trichrome to demonstrate their general structure. The H&E, Azan trichrome, van Gieson and Masson-Goldner trichrome staining scoring system was based on a standard protocol previously described (BURCK 1975). In addition, the methyl green-pyronin Y method (KURNICK 1955) was used to demonstrate the presence of plasma cells.

Histomorphometric study in H&E stained sections

In order to determine the capsule thickness according to the method described by EL-FADALY *et al.* (2014), the acinar outer diameters, tubule outer diameters and the thickness of the capsule and interlobar septae were measured. LG and SGTE measurements were taken. Additionally, we calculated the thickness of the tarsal plate. Histometric examination was performed on 16 eyelids (n = 16; 8 from females and 8 from males) and 8 glands (n = 8; 4 from females and 4 from males).

Thickness of the tarsal plate: Measurement of the tarsal plate thickness was carried out in 20 randomly chosen sections at 50x magnification (four animals/upper eyelid and lower eyelid).

Capsule thickness: The thickness of the capsule was measured in 60 randomly chosen parts from animals of each sex at 50x magnification (four animals/glands).

Interlobar septal thickenss: The interlobar septal thickness was measured in 60 randomly chosen parts from animals of both sexes under 50x magnification (four animals/glands).

Acinar and tubule diameters: The acinar and tubular diameters were measured in central fields at a 400x magnification. Measurements were taken at the widest diameter of the transversely cut acini and tubules from 60 randomly chosen specimens of both sexes (15 acini/animal/sex/gland and 15 tubules/animal/sex/gland).

Histological measurements of the gland structure were carried out using Axio Vision 4.8 (Carl Zeiss MicroImaging GmbH, Jena, Germany). Statistical analysis was performed using the Prism 3.0 program (GraphPad Software, Inc. San Diego, USA). Data were expressed as mean ± standard deviation (SD).

Histochemical analysis

The histochemical characterization of glycans, glycoconjugates and neutral glycoproteins was

Table 1

The morphometric parameters (mm) of the UE, LE, LG and SGTE in the red kangaroo. Values are expressed as mean ± standard deviations. UE – upper eyelid, LE – lower eyelid, LG – lacrimal gland, SGTE – superficial gland of the third eyelid

Parameters mean ± SD	UE	LE	LG	SGTE
Length (mm)	38.02 ± 7.3	38.02 ± 7.3	33.25 ± 3.1	20.64 ± 1.4
Width (mm)	19.36 ± 3.4	25.74 ±4.3	13.12 ± 2.7	13.91 ± 1.9
Thickness (mm)	3.93 ± 1.68	3.74 ± 1.14	5.25 ± 3.6	5.13 ± 2.1

detected using the periodic acid-Schiff method (PAS). The alcian blue pH 2.5 (AB pH 2.5) method was used for the identification of acid sialylated glycosaminoglycans. Hale's dialysed iron staining (HDI) was used to determine the presence of sulfated acid mucosubstances (SAM) and carboxylated acid mucosubstances (CAM). Sulfated acid mucosubstances and elastic fibers were identified using an aldehyde fuchsin staining (AF). The PAS, AB pH 2.5, AF and HDI staining scoring system was based on a standard protocol previously described (SPICER & HENSON 1967).

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

Electron microscopy investigation

The four LG samples (n = 4; 2 glands from females and 2 glands from males) and four SGTE samples (n = 4; 2 glands from females and 2 glands from males) were fixed in 2.5% glutaraldehyde dissolved in pH 7.4 0.1 M phosphate buffer for two weeks, and rinsed in a phosphate buffer. The material was then post-fixed in 4% OsO4 (osmium tetroxide) for 2 hours at room temperature. After re-rinsing in a phosphate buffer, the glands were dehydrated in an acetone series (from 30 to 100%). The dehydrated material was immersed in an Epon 812 epoxide resin. Blocks were cut into 70 nm sections with a diamond knife using a Leica Reichert Ultracut E microtome (Leica Microsystem Wetzlar GmbH, Germany). Preparations were assessed through the Zeiss EM 900 (Carl Zeiss, Jena, Germany) transmission electron microscope operating at 80 kV (KUBRAKIEWICZ *et al.* 2003). The study was performed in the Electron Microscopy Laboratory of the Wroclaw University.

Results

The histological and histochemical studies of UE, LE, TE, LG and SGTE in red kangaroo showed similarity between females and males. The Harderian gland was absent in red kangaroo.

Gross anatomy and topography

The morphometric parameters of the upper and lower eyelids, lacrimal gland and superficial gland of the third eyelid are shown in Table 1. Morphometric study showed that UE and LE were relatively bigger in males than in females (we are not able to show the significance of differences between females and males because of the small number of animals).

U p p e r a n d l o w e r e y e l i d. The UE and LE appeared to correspond to standard mammalian eyelid macromorphology (DYCE *et al.* 1996; GELLAT 1981; KEMPSTER *et al.* 2002). The UE was more extensive and more mobile than the LE. Long and thick eyelashes were found on the anterior palpebral margins of both eyelids, while shorter eyelashes were present on the lower eyelid (Fig. 1A-D).

T h i r d e y e l i d. The TE was located in the medial canthus of the eye. The TE resembled the letter T and was composed of cartilage, which consisted of a lower and an upper branch and a crossbar. The crossbar part of the TE was surrounded by the SGTE. The marginal part of the TE was thin and strongly pigmented (Fig. 1E).

L a c r i m a l g l a n d. The LG was flat, triangular in shape and light pink in colour. In gross appearance, it was uniform and undivided. It was positioned between the dorsal straight and the lateral straight muscles of the eyeball, at a dorsolateral angle to the periorbita (Fig. 1F). Its secretion was drained by small excretory ductules which opened into the superior conjunctival fornix.

S u p e r f i c i a l g l a n d o f t h e t h i r d e y e l i d. The SGTE was oval and light pink. Similarly to the LG, it was uniform and undivided. It was located between the medial straight and ventral straight muscle and was partially covered by the ventral oblique muscle of the eyeball (Fig. 1G).

Light Microscopy Observations

Histological findings

The histomorphometric parameters of the LG and SGTE capsule and interlobar septae thickness, acinar and tubule outer diameters are shown in Table 2 and Fig. 9.

U p p e r a n d l o w e r e y e l i d s. The UE and LE contain an anterior and posterior surface. The anterior surface of the eyelids is made of thin and delicate skin covered with short hairs, with a few prominent tactile hairs (Fig. 1A,C). In our study, the skin was covered by a keratinizing stratified squamous epithelium (Fig. 2A). Dense irregular connective tissue was found directly beneath the epidermis. Numerous scattered melanocytes were present near the stratum basale (Fig. 3B). Long and thick eyelashes arranged in 2-3 rows grew from the anterior palpebral margins. Sebaceous glands and ciliary glands, which are modified sweat glands located deep in the follicle, issued secretions into the hair follicles (Figs 2A, C and 3C). The muscle layer was situated deep in the dermis and consisted of bundles of the orbicularis oculi muscle, levator anguli oculi medialis and malaris muscles (Fig. 2A). Under the muscle layer was a layer of connective tissue with blood vessels and

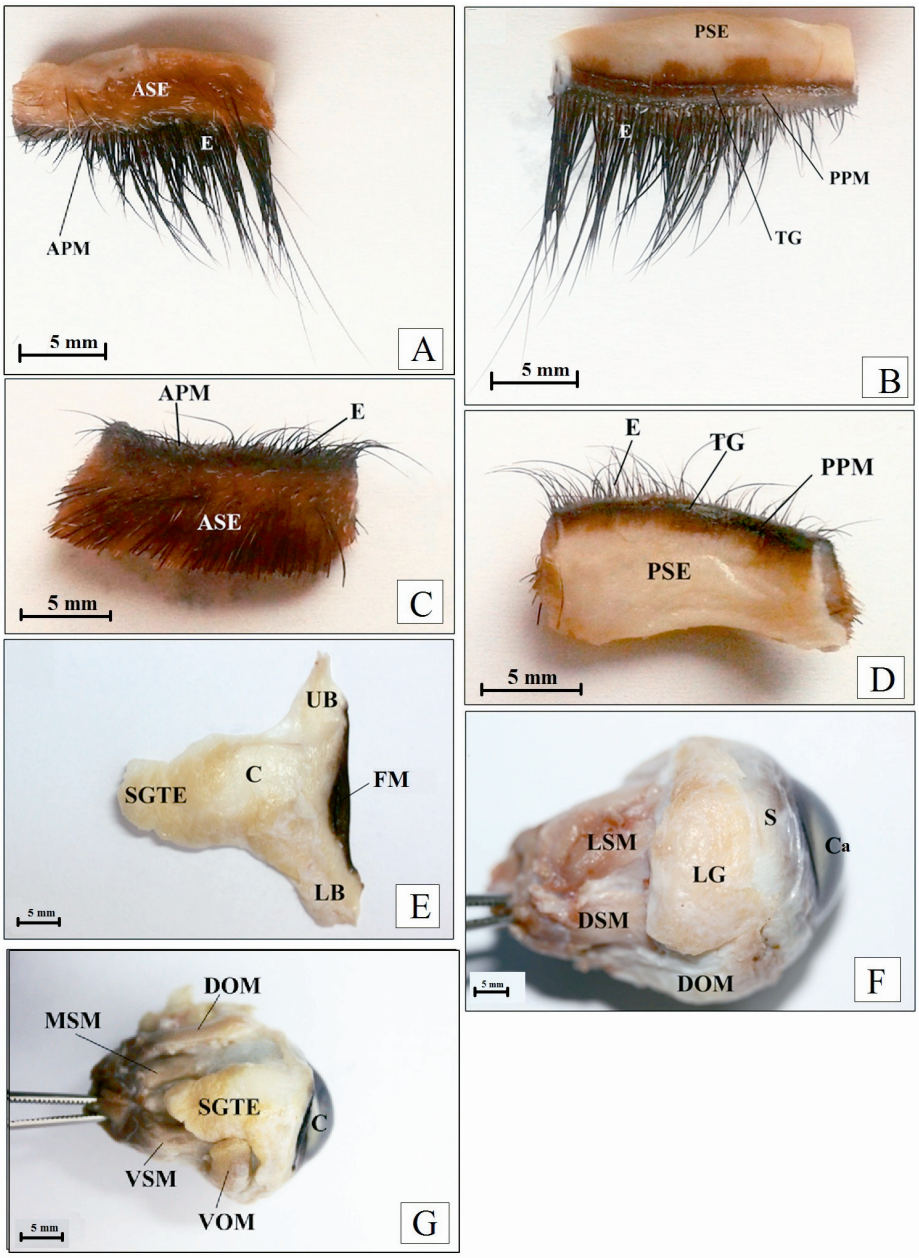

Fig. 1. Macrograph of the UE, LE, LG and SGTE with TE in red kangaroo: A, B – upper eyelid. Bar = 5 mm; C, D – lower eyelid. Bar = 5 mm; E – third eyelid. Bar = 5 mm; F – lacrimal gland. Bar = 5 mm; G – superficial gland of the third eyelid. Bar = 5 mm. ASE – anterior surface of eyelids, APM – anterior palpebral margins, E – eyelashes, PSE – posterior surface of eyelids, PPM – posterior palpebral margins, TG – tarsal gland, UB – upper branch, LB – lower branch, C – crossbar, FM – free margin, Ca – cornea, DOM – dorsal oblique muscle, LSM – lateral straight muscle, DSM – dorsal straight muscle, VOM – ventral oblique muscle, MSM – medial straight muscle, VSM – ventral straight muscle, LG – lacrimal gland, SGTE – superficial gland of the third eyelid.

Table 2

The histometric parameters (μm) of the LG and SGTE in the red kangaroo. Values are expressed as mean ± standard deviations. LG – lacrimal gland, SGTE – superficial gland of the third eyelid, M – male, F – female

Parameters mean ± SD	LG		SGTE	
	M	F	M	F
Thickness of the capsule (μm)	535.4 ± 48.3	558.4 ± 59.1	228.2 ± 21.7	278.1 ± 23.4
Thickness of the interlobares septae (μm)	143.6 ± 13.1	166.7 ± 12.9	150.2 ± 12.7	168.2 ± 13.3
Outer diameters of the acini (μm)	44.3 ± 2.7	50.1 ± 2.3	26.4 ± 2.5	37.5 ± 3.3
Outer diameters of the tubules (μm)	88.2 ± 9.5	95.7 ± 4.6	79.7 ± 5.1	101.2 ± 5.8

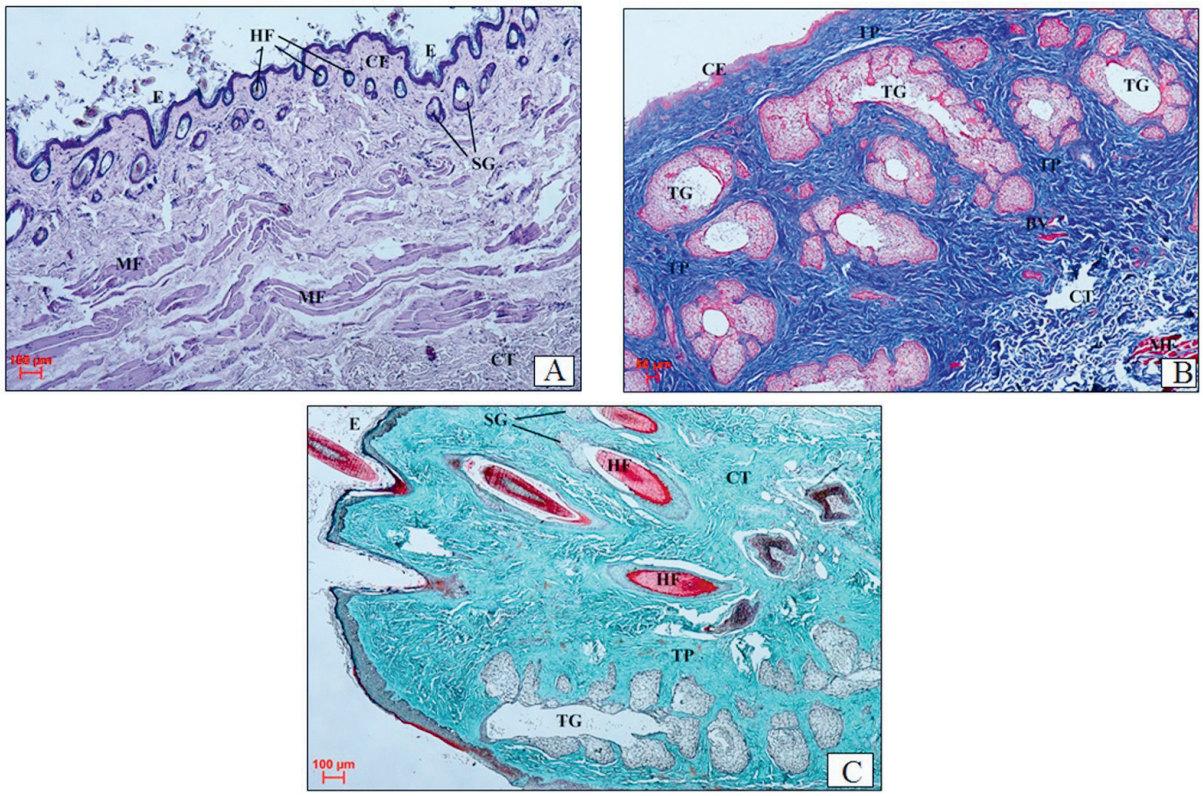

Fig. 2. Light micrograph of UE in red kangaroo: A – H&E stain. Bar = 100 µm; B – azan trichrome stain. Bar = 50 µm; C – Masson-Goldner stain. Bar = 100 µm.
E – epithelium, HF – hair follicle, CF – collagenous fibres, SG – sebaceous glands, MF – muscle fibres, CT – connective tissue, CE – conjunctival epithelium, TP – tarsal plate, BV – blood vessels.

nervous fibres (Fig. 3D). The tarsal plate and tarsal glands were located within the connective tissue layer. The tarsal plate consisted of dense fibrous connective tissue (Fig. 2B). The upper tarsal plate was thicker (1961.19µm ± 63.4) than the lower tarsal plate (877.21µm ± 52.7). The tarsal glands were elongated vesicular glands. The tarsal gland ducts had a stratified squamous epithelium lining and their openings were located in the posterior palpebral margins of both eyelids (Fig. 2C). The posterior surface of the eyelids was lined with a non-keratinized stratified squamous epithelium (Fig. 2B). The numerous goblet cells in the palpebral and bulbar conjunctiva zones (defined as non-lymphoid conjunctiva or non-lymphoid region) of the UE and within LE were observed (Fig. 3A). Under the conjunctival epithelium, there was loose connective tissue which contained several blood vessels. Organized lymphoid follicles were observed only in the lymphoid conjunctiva (defined as lymphoid region) in the LE (Fig. 3E). Diffuse lymphocytes were not found in either eyelid.

T h i r d e y e l i d. The TE cartilage was directly surrounded by a thick layer of elastin and collagen fibres, and then encircled by abundant adipose tissue (Fig. 4A). The TE cartilage was composed of hyaline tissue with numerous chondrocytes and a smaller amount of intercellular substance than in hyaline cartilage. Chondrocytes in deep parts of the cartilage were oval in shape and were mostly single. Sometimes they formed an isogenic group composed of 2-3 cells (Fig. 4B,C). The conjunctival and ocular surfaces of the TE had a non-keratinized stratified squamous epithelium (Fig. 4D). Numerous goblet cells were observed within the non-keratinized multilayer epithelium of the TE (Fig. 4D).

L a c r i m a l g l a n d. The LG was a multilobar tubuloacinar gland with a predominance of acini. It was covered by a thin connective tissue capsule, consisting of collagen and elastic fibres (Fig. 5A). The connective tissue of the capsule penetrated into the glandular parenchyma and formed septa, which divided the gland into large and smaller lobes (Fig. 5A). The lobes were composed with acini and tubules. The acini containing a small lumen were composed of tall conical cells surrounded by basal myoepithelial cells (Fig. 5C). The round acinar nuclei were located in the basal part of the cells. These cells had basophilic cytoplasm (Fig. 5C). The tubules with a large lumen were composed of a single cubic cell layer with round nuclei, located in the basal areas of those cells (Fig. 5C). The main ducts had a simple cuboi-

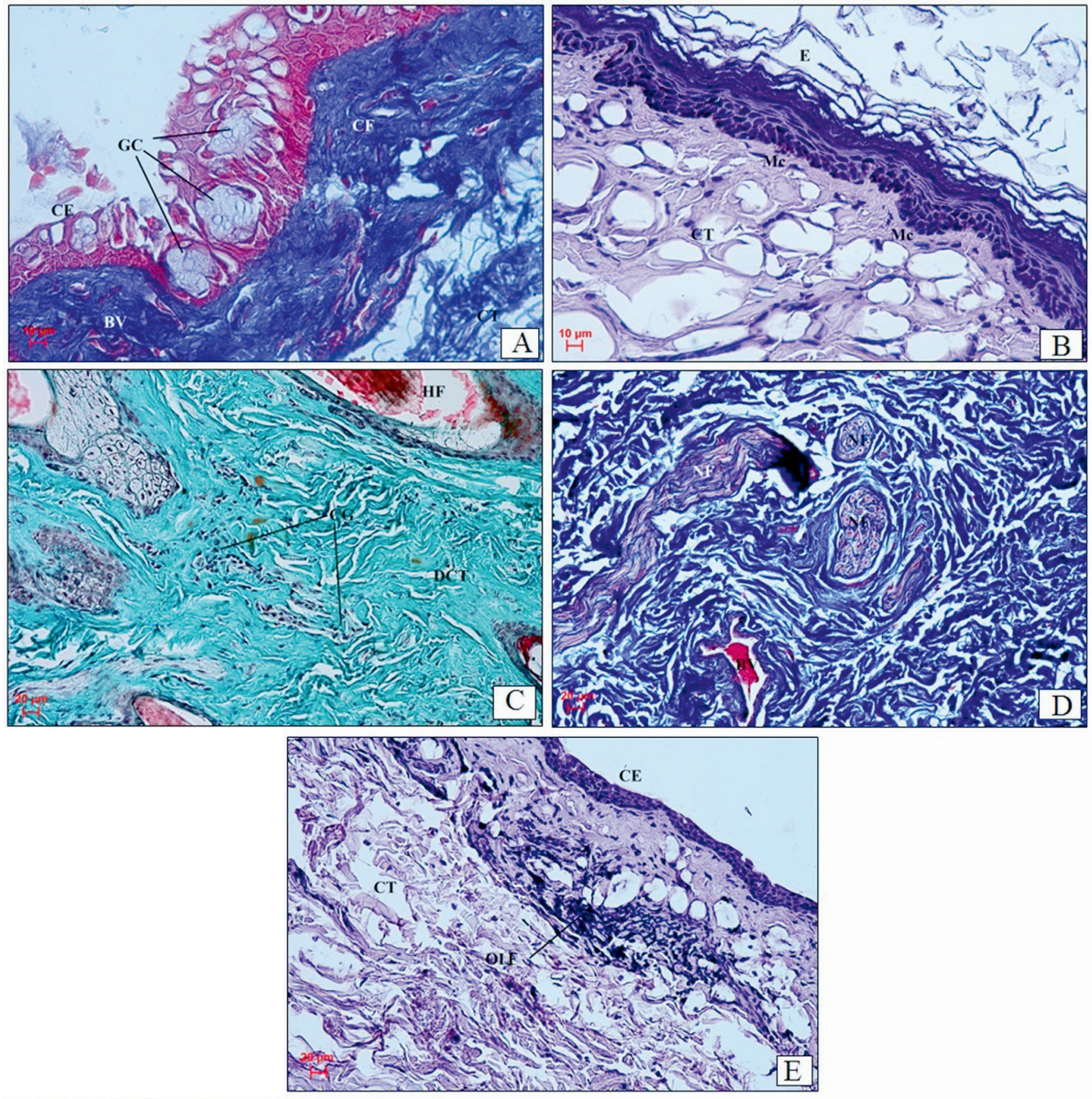

Fig. 3. Light micrograph of LE in red kangaroo: A – azan trichrome stain. Bar = 10 µm; B – H&E stain. Bar = 10 µm; C – Masson-Goldner stain. Bar = 20 µm; D – azan trichrome stain. Bar = 20 µm; E – H&E stain. Bar = 20 µm. E – epithelium, HF – hair follicles, CG – ciliary glands, Mc – melanocytes, CF – collagenous fibres, GC – goblet cells, CT – connective tissue, CE – conjunctival epithelium, NF – nervous fibres, BV – blood vessels, DCT – dense connective tissue, OLF – organized lymphoid follicle.

dal lining. The MGP Y staining demonstrated the presence of numerous plasma cells with a characteristic dark-pink nucleus and pink cytoplasm localized in the glandular interstitium (Fig. 5E).

Superficial gland of the third eyelid. The SGTE had a multilobar tubuloacinar structure. The SGTE was surrounded by a thin connective tissue capsule, which divided the gland structure into lobes, consisting of acini and tubules (Fig. 5B). Numerous single adipocytes and single blood vessels were visible within the interlobar septae (Fig. 5B). The acini with a small lumen were made of tall conical secretory cells with an eosinophilic cytoplasm. The rounded nuclei of the acinar cells were located close to the base of the cells (Fig. 5D). The tubules had a cuboidal epithelium with an eosinophilic cytoplasm. The nuclei of the tubular cells were oval in shape and located in the central part of these cells (Fig. 5D). The main excretory duct was lined with a stratified columnar epithelium with single goblet cells. The MGP Y staining in SGTE revealed the presence of numerous plasma cells with a characteristic dark-pink nucleus and pink cytoplasm (Fig. 5F).

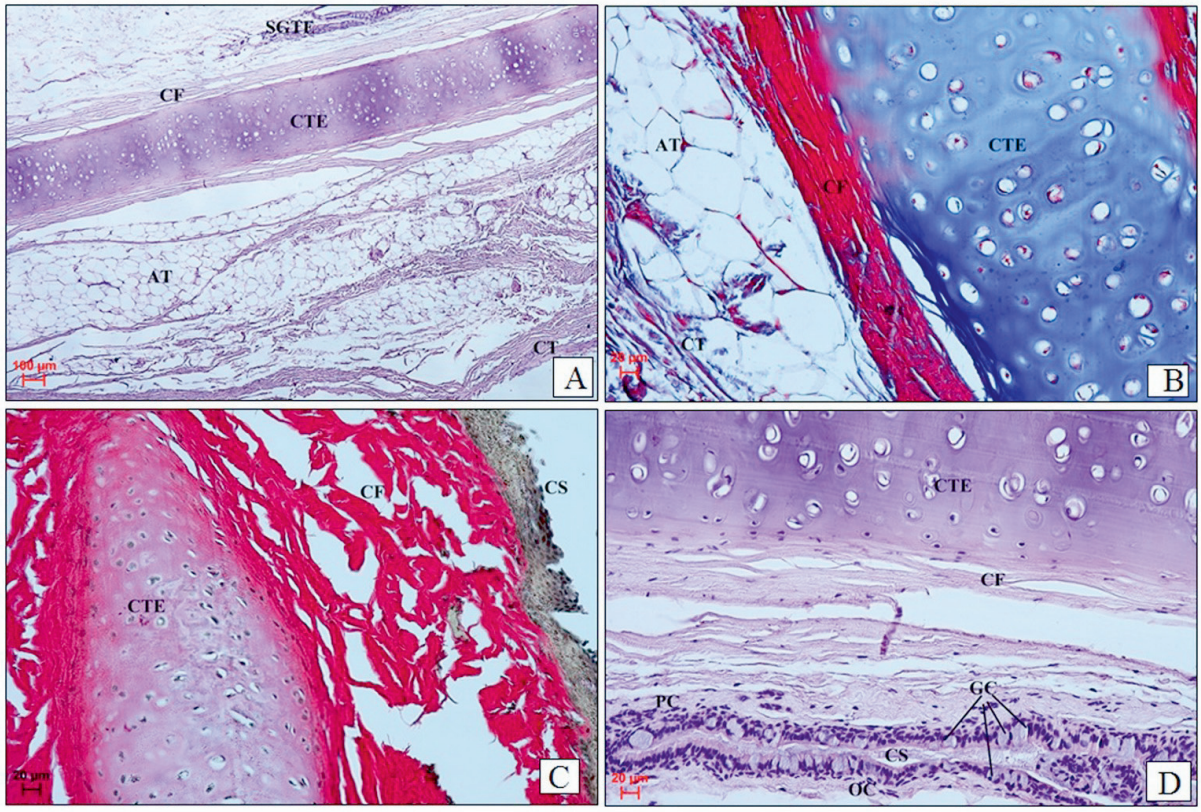

Fig. 4. Light micrograph of SGTE in red kangaroo: A – H&E stain. Bar = 100 µm; B – azan trichrome stain. Bar = 20 µm; C – van Gieson stain. Bar = 20 µm; D – H&E stain. Bar = 20 µm.
CF – collagenous fibres, CTE - cartilage of the third eyelid, SGTE – superficial gland of the third eyelid, AT – adipose tissue, OS – ocular surface, CS – conjunctival surface, GC – goblet cells, CT – connective tissue, PC – palpebral conjunctiva, OC – ocular conjunctiva.

Table 3

Histochemical analysis of the LG and SGTE in red kangaroo. UE – upper eyelid, LE – lower eyelid, LG – lacrimal gland, SGTE – superficial gland of the third eyelid, SG – sebaceus glands, TG – tarsal glands, CG – ciliary glands, GC – goblet cells, A – acini, T – tubules, PAS – period acid-Schiff, AB pH 2.5 – Alcian blue pH 2.5, HDI – Hale's dialysed iron, AF – aldehyde fuchsin, SAM – sulfated acid mucopolysaccharides, CAM – carboxylated acid mucopolysaccharides

Gland		PAS	AF		AB pH 2.5	HDI	
			CAM	SAM		CAM	SAM
UE	SG	–	x		–	x	
	TG	–	x		–	x	
	CG	–/+	x		–/+	x	
	GC	+++	x		+++	x	
LE	SG	–	x		–	x	
	TG	–	x		–	x	
	SG	–/+	x		–/+	x	
	GC	+++	x		+++	x	
LG	A	–/+	–		+/++	++	–
	T	++	–		–/+	++	–
SGTE	A	–/+	–		+/++	+ + +++	–
	T	–/+	–		+/++	+ + +++	–

Histochemical findings

The histochemical characteristics of the upper and lower eyelids, lacrimal gland and superficial gland of the third eyelid are shown in Table 3.

Upper and lower eyelid. The presence of PAS negative sebaceous and tarsal glands was found (Fig. 6A, E). The goblet cells located in the conjunctival epithelium were strongly PAS positive (Fig. 6C) which indicated the presence of neutral glycoproteins, glycolipids and phospholipids. Sebaceous and tarsal glands did not stain with AB pH 2.5 (Fig. 6B, F), while goblet cells of the conjunctival epithelium stained strongly, which indicated the presence of acid sialylated glycosaminoglycans in those cells (Fig. 6D). Staining using PAS and AB pH 2.5 gave weak positive reactions in the ciliary glands (Fig. 6A).

Lacrimal gland. PAS staining showed acinar cells to have a weak positive reaction and tubular cells to have a mild positive reaction (Fig. 7A), indicating that these cells had some amount of neutral glycoproteins, glycolipids and phospholipids. The AB pH 2.5 staining demonstrated the presence of acid sialylated glycosaminoglycans. Histochemical analysis using AB pH 2.5 showed a mild positive reaction in the acini and tubules (Fig. 7C). The acinar and tubular cells (dark blue in color) gave a mild

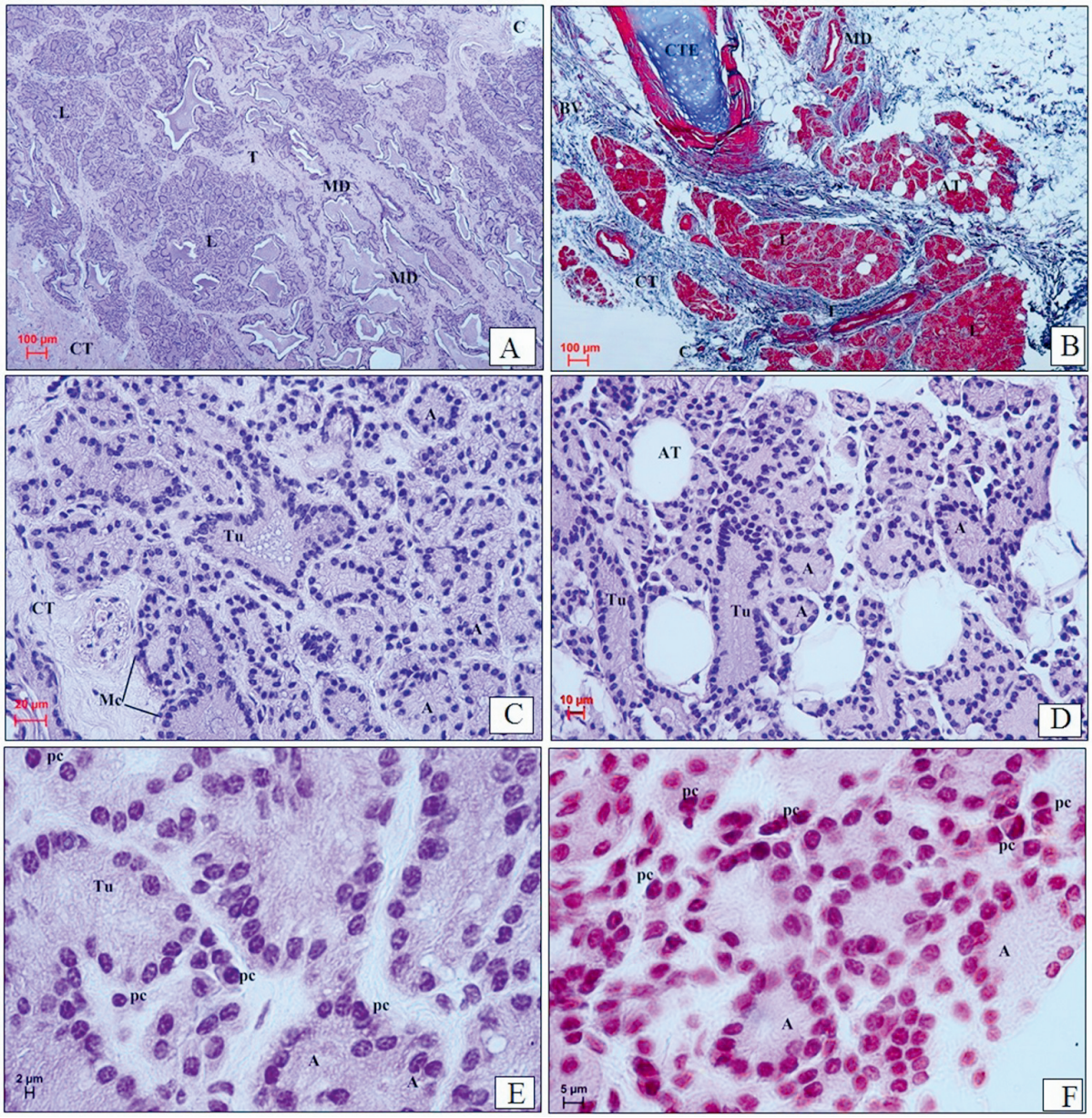

Fig. 5. Light micrograph of LG (A, C, E) and SGTE (B, D, F) in red kangaroo: A – H&E stain. Bar = 100 µm; B – azan trichrome stain. Bar = 100 µm; C – H&E stain. Bar = 20 µm; D – H&E stain. Bar = 20 µm; E – MGP Y stain. Bar = 2 µm; F – MGP Y stain. Bar = 5 µm.
C – capsule, CT – connective tissue, L – lobus, BV – blood vessels, AT – adipose tissue, T – trabeculae, A – acini, Tu – tubules, MD – main duct, Mc – myoepithelial cells, pc – plasma cells.

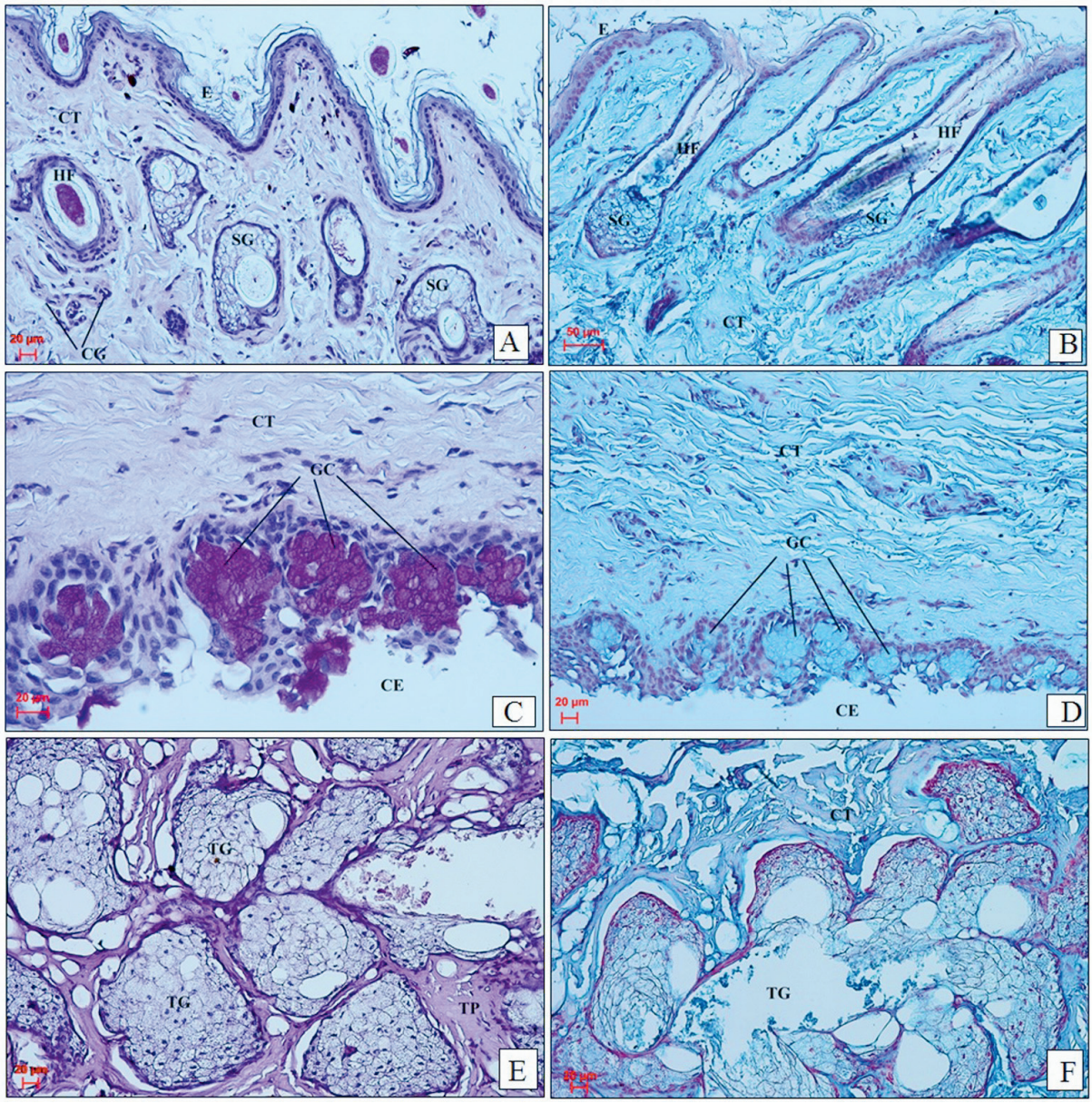

Fig. 6. Light micrograph (histochemistry) of the UE (A, B, C) and LE (D, E, F) in red kangaroo: A – PAS stain. Bar = 20 µm; B – AB pH 2.5 stain. Bar = 50 µm; C – PAS stain. Bar = 20 µm; D – AB pH 2.5 stain. Bar = 20 µm; E – PAS stain. Bar = 20 µm; F – AB pH 2.5 stain. Bar = 20 µm.
E – epithelium, HF – hair follicle, GC – goblet cells, SG – sebaceous gland, CG – ciliary glands, CT – connective tissue, CE – conjunctival epithelium, TP – tarsal plate.

positive reaction using the HDI method, indicating the presence of CAM (Fig. 7E). We observed a negative acinar and tubular reaction in AF staining (Fig. 7G). Histochemical studies using PAS, AB pH 2.5, AF and HDI showed that the LG in the red kangaroo produced a mucoserous secretion.

Superficial gland of the third eyelid. The PAS staining method demonstrated the presence of isolated (scarce) secretory cells with a weak positive reaction (Fig. 7B). The AB pH 2.5 staining demonstrated the presence of acid sialylated glycosaminoglycans. Similarly to LG, the histochemical analysis of SGTE using AB pH 2.5 indicated a mild positive reaction in the acini and tubules (Fig. 7D). The HDI method showed that numerous acinar and tubular cells had a strong positive reaction, while single secretory cells had a mild positive reaction, revealing the presence of CAM in these cells (Fig. 7F). AF staining showed a negative reaction in the acini and tubules (Fig. 7H). Histochemical studies using PAS, AB pH 2.5, AF and HDI showed that the SGTE secretion in the red kangaroo had a mucoserous character.

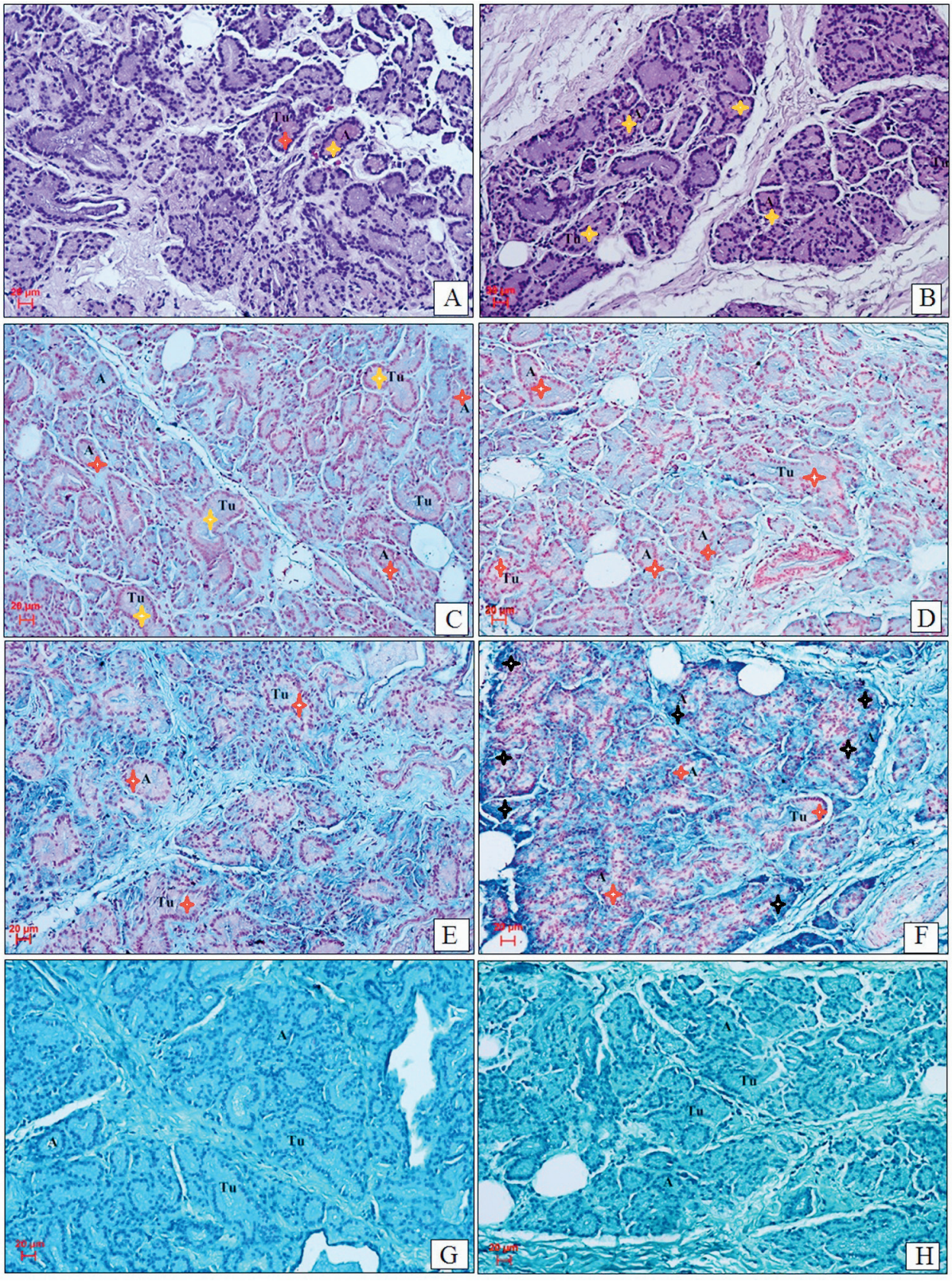

Fig. 7. Light micrograph (histochemistry) of the LG (A, C, E, G) and SGTE (B, D, F, H) in red kangaroo: A, B – PAS stain. Bar = 20 µm; C, D – AB pH 2.5 stain. Bar = 20 µm; E, F – HDI stain. Bar = 20 µm; G, H – AF stain. Bar = 20 µm.
A – acini, Tu – tubules, yellow asterisk – weakly reaction, red asterisk – middle reaction, black asterisk – strong reaction.

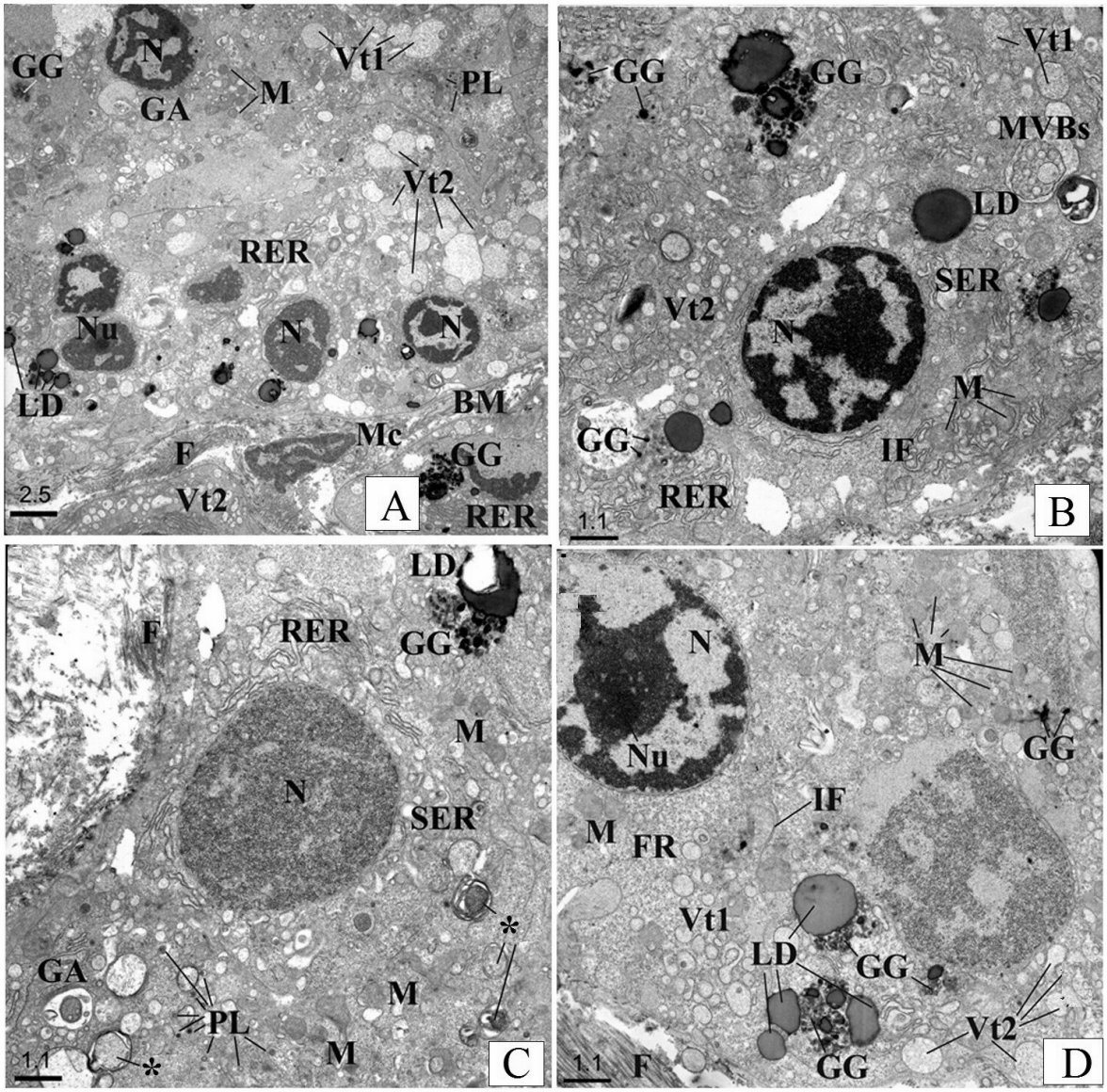

Fig. 8. Overview electron micrograph of the LG (A, B) and SGTE (C, D) cells in red kangaroo: A – Note of the secretory cells with visible myoepithelial cells. Bar = 2.5 μm; B – Note secretory cells with the nuclei of acini and vesicles type 1 and type 2. Bar = 1.1 μm; C – Note secretory acini with extensive membrane structures arranged in concentric lamellae. Bar = 1.1 μm; D – Note secretory cells with the nuclei of tubules and big lipid drops. Bar = 1.1 μm.
N – nucleus of the cell, Nu – nucleolus, RER – rough endoplasmic reticulum, M – mitochondria, Mc – myoepithelial cells, SER – smooth endoplasmic reticulum, GG – glycogen granules, LD – lipid drops, BM – basement membrane, F – filaments, GA – Golgi apparatus, IF – intermediate filaments, PL – primary lysosomes, FR – free ribosomes, Vt1 – vesicles type 1, Vt2 – vesicle type 2, MVBs –multivesicular bodies, * – extensive membrane structures arranged in concentric lamellae.

Electron microscopy investigation

The TEM study showed that the secretory cells of the LG and SGTE in the red kangaroo had a similar ultrastructural appearance. The nuclei in all the acini were large, spherical and located basally. They contained visible nucleoli and considerable amounts of heterochromatin (Fig. 8A, D), whereas the nuclei of the tubules were oval (Fig. 8D). These gland cells were frequently mononuclear. The rough endoplasmic reticulum (rER) surrounding the nucleus and often showing continuity with the nuclear envelope was present in all glands (Fig. 8A-C). In the LG, the smooth endoplasmic reticulum (sER) appeared as small fenestrated cisternae which formed concentric layers located between the supranuclear region of the secretory cells and the lipid droplets (Fig. 8B). The LG had numerous small and oval mitochondria, frequently with transverse cristae and a dense matrix (Fig. 8A, B). Numerous large mitochondria were located between the secretory vesicles and the rER in the SGTE (Fig. 8C). The Golgi apparatus was located

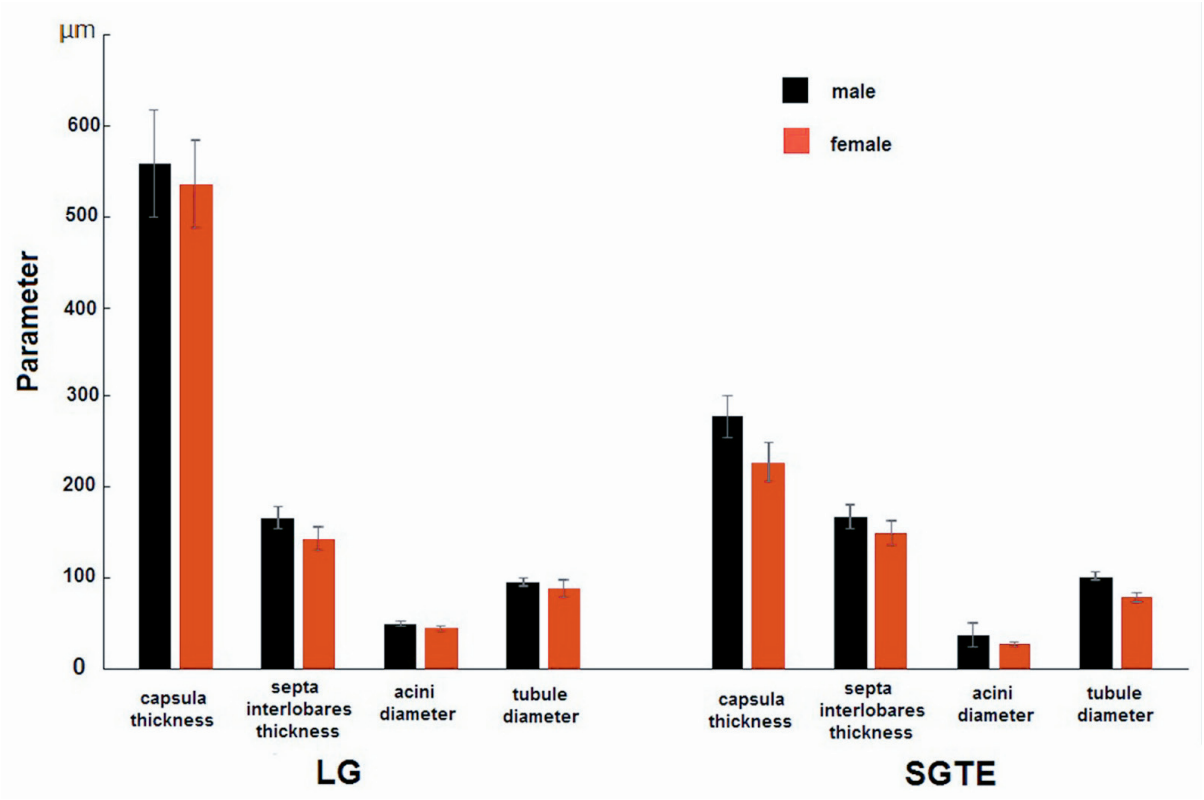

Fig. 9. Histometric parameters (µm) of the LG and SGTE in red kangaroo. Values are expressed as mean ± standard deviations. LG – lacrimal gland, SGTE – superficial gland of the third eyelid.

near the nucleus and was composed of low and flat cistern layers, narrow vesicles and vacuoles (Fig. 8A). In the secretory cells, large lipid droplets were found in close vicinity with glycogen granules and secretory vesicles (Fig. 8A, D). The lipid droplets were more numerous in the LG than in the SGTE. Small primary lysosomes and numerous glycogen granules were present within the cytoplasm in the LG and SGTE secretory cells (Fig. 8A-D). The sparse extensive membrane structures were arranged in concentric lamellae, and were found only in the SGTE cells (Fig. 8C). Intermediate filaments were also present within the cytoplasm in the LG and SGTE (Fig. 8B, D). Two types of secretory vesicles (Vt1 and Vt2) were detected in the cytoplasm of these glands in the acini and tubules (Fig. 8A, B, D). Type 1 vesicles had high electron-density and contained varying amounts of homogenous material. Type 1 vesicles were bigger in the LG than in the SGTE (Fig. 8A, B, D). Type 2 vesicles had lower electron-density with a fine-grained content. Type 2 secretory vesicles were more numerous than type 1 vesicles in both glands. The sparse multivesicular bodies (MVBs), also referred to as late endosomes, were observed in the cytoplasm of the LG cells (Fig. 8B).

Discussion

The structures of the LE, UP, TE, LG and SGTE of the red kangaroo are similar to those of other animals (ALDANA MARCOS *et al.* 2002; DJERIDANE 1992; DYCE *et al.* 1996; GARGIULO *et al.* 1999; GELLAT 1981; KEMPSTER *et al.* 2002; MARTIN *et al*. 1988; PRADIDARCHEEP *et al*. 2003; WEAKER 1981), and they have not been described to date. In the present study, some new features were discovered in the macroscopic and microscopic structures of the accessory organs of the red kangaroo.

The UE and LE in the red kangaroo have a similar anatomical and histological structure common to all mammals (DYCE *et al*. 1996; GELLAT 1981; NICKEL *et al.* 2004). However, some differences between red kangaroo and other mammals were observed, especially in eyelid structure, connected with conjunctiva-associated lymphoid tissue (CALT). Eyelashes in red kangaroo are present in both eyelids similar as in small domestic ruminants and horses (NICKEL *et al.* 2004). On the other hand, CONSTANTINESCU & MOORE (1998) showed that eyelashes are present in the upper eyelid in the dog, while cats did not have eyelashes in

either eyelid. In the red kangaroo, numerous sebaceous and ciliary glands issuing secretions into the eyelash follicles were present. These glands were examined in humans, primates (e.g. rhesus monkey, *Macaca mulatta*) and non-primate mammals (e.g. pig, cow, horse and carnivores) (IKEDA 1953; STEPHENS *et al.* 1989; STOECKELHUBER *et al.* 2003, 2004; YASUI *et al.* 2006). The posterior palpebral margins in the red kangaroo and other mammals are lined with multiple tarsal glands which appear as yellow columns positioned parallel to one another and perpendicular to the eyelid margins (CONSTANTINESCU & MOORE 1998; FAHMY *et al.* 1971; REHOREK *et al.* 2010a). As reported by REHOREK *et al.* (2010a), tarsal glands do not occur in fetal species of bats. Based on histological findings, KAGEYAMA *et al.* 2006 in Japanese monkeys and BAYRAKTAROGLU *et al*. (2011) in the ostrich (*Struthio camelus*) showed differences in conjunctival epithelium between the non-lymphoid and lymphoid conjunctiva (defined as non-lymphoid and lymphoid region). These authors described that the non-lymphoid region had numerous goblet cells, while in the lymphoid region the epithelium does not contain goblet cells, although aggregated and solitary lymphoid follicles do occur (KLEĆKOWSKA-NAWROT *et al.* 2016). This study showed the presence of goblet cells in the palpebral and bulbar conjunctiva zones (non-lymphoid conjunctiva) of both eyelids, but numerous goblet cells were identified in the palpebral conjunctiva zone of the LE, which were absent in the lymphoid region. Diffuse lymphocytes were absent in UE and LE, which is typical for birds (BAYRAKTAROGLU *et al.* 2011; KLEĆKOWSKA-NAWROT *et al.* 2016; VAN GINKEL *et al.* 2012). According to studies on chinchilla (*Chinchilla lanigera*; VOIGT *et al.,* 2012) and guinea pig (GASSER *et al.,* 2011), numerous goblet cells were located in the bulbar and palpebral conjunctiva zones and a few goblet cells were present in the lymphoid region in the UE and LE. Our study revealed the presence of numerous organized lymphoid follicles only within LE lymphoid conjunctiva. According to KNOP and KNOP (2000) and SIEBELMANN *et al*. (2013), in humans the CALT is present as organized lymphoid follicles within both eyelids, however in LE they are more abundant. The CALT in humans is present also as diffuse lymphoid tissue in the upper and lower eyelids, which was not found in red kangaroo. Interestingly, the CALT in rats and mice has very little lymphoid tissue which does not show organized lymphoid follicles under physiological state, and has very few diffusely interspersed lymphoid cells (CHODOSH *et al.* 1998; KLEĆKOWSKA-NAWROT *et al.* 2016; KNOP & KNOP 2005). CALT was also described as solitary or aggregate lymphoid follicles in Angora goats, Japanese monkeys and domestic animals (ASTI *et al.* 2000; BAYRAKTAROGLU & ASTI 2009; CAIN & PHILIPS 2008; CONSTANTINESCU & MOORE 1998; KAGEYAMA *et al.* 2006; SCHLEGEL *et al.* 2003).

The TE is a prominent semilunar fold of the conjunctiva. In the red kangaroo, it was located in the medial angle of the eye similarly as in elasmobranch fish, amphibians, reptiles, birds and mammals (except humans) lemurs and lorisoid primates (ALDANA MARCOS *et al.* 2002). The TE in the red kangaroo resembles the letter T. It has a similar shape in carnivores, small ruminants and one-humped camel (*Camelus dromedarius*) (AL-RAMADAN & ALI 2012; CONSTANTINESCU & MCCLURE 1990; KLEĆKOWSKA-NAWROT & DZIĘGIEL 2007; SCHLEGEL *et al*. 2001; THOMPSON 1958). According to SCHLEGEL *et al*. (2001), KLEĆKOWSKA-NAWROT and DZIĘGIEL (2007), KLEĆKOWSKA-NAWROT *et al.* (2015a) and KLEĆKOWSKA-NAWROT *et al*. (2015b), the TE has an anchor shape in the pig, alpaca and the European bison. The marginal part of the TE in the red kangaroo was thin and very strongly pigmented. Similar results were also reported by AL-RAMADAN and ALI (2012) in the one-humped camel and by KLEĆKOWSKA-NAWROT *et al.* (2015b) in the European bison. As stated by KEMPSTER *et al.* (2002), the TE is large and thick in the koala. Histological analysis showed that the TE cartilage in the red kangaroo is surrounded by abundant adipose tissue with elastic and collagen fibres. This cartilage was composed of hyaline tissue in the red kangaroo. A similar structure was described by BISARIA and BISARIA (1978), KEMPSTER *et al.* (2002), KLEĆKOWSKA-NAWROT (2005), KLEĆKOWSKA-NAWROT *et al.* (2015a), KLEĆKOWSKA-NAWROT *et al*. (2015b), NICKEL *et al*. (2004), and SCHLEGEL *et al.* (2001) in the pig, dog, koala, rat, and domestic ruminants, respectively. However, in the one-humped camel, cat and horse the TE cartilage was composed of elastic tissue (AL-RAMADAN & ALI 2012; CONSTANTINESCU & MOORE 1998; and SCHLEGEL *et al.* 2001). The conjunctival and ocular surface of the TE in the red kangaroo, similarly to the dog and wild ruminants, consisted of non-keratinized stratified squamous epithelium with numerous goblet cells (AL-RAMADAN & ALI 2012; CAZACU 2010; KLEĆKOWSKA-NAWROT *et al.* 2015a; KLEĆKOWSKA-NAWROT *et al.* 2015b).

As in other mammals, the LG in the red kangaroo is located between the dorsal straight and the lateral straight muscles of the eyeball, at a dorsolateral angle to the periorbit (ABBASI *et al.* 2014; ALDANA MARCOS *et al*. 2002; HENKER *et al.* 2013; KEMPSTER *et al*. 2002; KLEĆKOWSKA-NAWROT *et al*. 2015a; PINARD *et al.* 2003; SHADKHAST & BIGHAM 2010). The LG in the ex-

amined animals was uniform and undivided, flat, triangular and light pink. Similar observations were made in pigs in the prenatal period by KLEĆKOWSKA-NAWROT and DZIĘGIEL (2008). The LG in sheep was flattened, oval and red (GARGIULO *et al*. 1999) and ovoid and light brown in the donkey (ALSAFY 2010), while in the one-humped camel (*Camelus dromedarius*) the LG appeared to be flattened, elongated and had an irregular structure (MOHAMMADPOUR 2008). In the Iranian River buffalo, the LG was flattened, oval, pale yellow and lobulated (SHADKHAST & BIGHAM 2010). HENKER *et al*. (2013) reported that the LG in pigs had a soft and pale structure. On the other hand, this gland was triangular with a cobble-stoned proximal part and a smooth-appearing distal portion that appeared smooth in the American bison and cattle (PINARD *et al.* 2003). KEMPSTER *et al.* (2002) found the LG in the koala (*Phascolarctos cinereus*) had a slightly lobulated surface and was embedded in fat. On average the LG in the red kangaroo was relatively larger than the SGTE. Differences in the size between the LG and SGTE were also observed in the American bison, alpaca (*Vicugna pacos*) and pig (KLEĆKOWSKA-NAWROT 2005; KLEĆKOWSKA-NAWROT *et al.* 2015a; PINARD *et al.* 2003). Clearly visible differences in the size of the LG were also observed among different mammalian species (ABBASI *et al.* 2014; ALSAFY 2010; HENKER *et al.* 2013; KEMPSTER *et al.* 2002; MOHAMMADPOUR 2009; SHADKHAST & BIGHAM 2010). Due to the small study group, we did not correlate the size of the LG with respect to sex. However, differences in the size of the LG between males and females were reported in the dog by CABRAL *et al.* (2005), in pig fetuses by KLEĆKOWSKA-NAWROT (2005), in roe deer by KLEĆKOWSKA-NAWROT *et al.* (2013), in alpaca by KLEĆKOWSKA-NAWROT *et al.* (2015a), and in rats and mice by CORNELL-BELL *et al.* (1985) and EL-FADALY *et al*. (2014). In histological examination the LG in red kangaroo is a multilobar tubuloacinar gland with a predominance of acini with numerous plasma cells, which is typical in animals without a Harderian gland. Each lobule in the LG of the red kangaroo is made up of several acini with tall conical secretory cells, whereas in the dog and Philippine Water buffalo (*Bubalus bubalis*) the acini are tall pyramidal or columnar cells (MAALA *et al.* 2007; MARTIN *et al.* 1988). The cytoplasm of the acinar cells in the LG in the red kangaroo, alpaca, American bison, Iranian River buffalo and albino rats of the Wistar strain is basophilic (EL-FADALY *et al.* 2014; KLEĆKOWSKA-NAWROT *et al.* 2015a; PINARD *et al.* 2003; SHADKHAST & BIGHAM 2010). Our study showed that the LG secretion in the red kangaroo had a mucoserous character. Similar results were obtained in the study on musk shrew (*Suncus murinus*), domestic ruminants, the alpaca and the dog (GARGIULO *et al.* 1999; KLEĆKOWSKA-NAWROT *et al.* 2015a; MARTIN *et al.* 1988; SAKAI 1989).

The SGTE of the red kangaroo was located between the medial straight and ventral straight muscle and was partially covered by the ventral oblique muscle of the eye. A similar location of this gland was demonstrated in other mammal species (AL-RAMADAN & ALI 2012; CAZACU 2010; CONSTANTINESCU & MCCLURE 1990; KLEĆKOWSKA-NAWROT & DZIĘGIEL 2007; KEMPSTER *et al.* 2002; KLEĆKOWSKA-NAWROT *et al*. 2013; KLEĆKOWSKA-NAWROT *et al.* 2015a; MOHAMMADPOUR 2009; NUYTTENS & SIMOENS 1995; PINARD *et al*. 2003; SCHLEGEL *et al.* 2001). Similarly to the LG, the SGTE was uniform and undivided. The gland was light pink and oval similarly to that of wild ruminants (AL-RAMADAN & ALI 2012; KLEĆKOWSKA-NAWROT *et al*. 2013; KLEĆKOWSKA-NAWROT *et al.* 2015a). KEMPSTER *et al.* (2002) showed that the SGTE in the koala had a lobulated surface. In cattle, that gland had a lobulated or cobblestoned appearance (PINARD *et al.* 2003). Interestingly, REHOREK *et al.* (2010b) examined the anterior orbital glands in the fat-tailed dunnart (*Sminthopsis crassicaudata*, Dasyuridae: Marsupiala), and showed the presence of one conjunctival gland and three non-conjunctival glandular units: two non-conjunctival glands located superficially in the third eyelid, while the third non-conjunctival gland was present deeper within the connective tissue. Based on the above mentioned study in the fat-tailed dunnart two non-conjunctival glands were superficial glands of the third eyelid, while a third (glandular units) was a deep gland of the third eyelid (Harderian gland) according to Anatomical Veterinary Terminology (2002) and NAV (2012) and NICKEL *et al.* (2004). However, in red kangaroo the Harderian gland was absent. In the red kangaroo, the SGTE was smaller than the LG. This is contrary to the finding of KEMPSTER *et al*. (2002) who noted that the SGTE was larger than the LG in the koala. The SGTE tended to be smaller in females than in males , what was also shown in pig fetuses by KLEĆKOWSKA-NAWROT (2005) and in roe deer by KLEĆKOWSKA-NAWROT *et al.* (2013). According to REHOREK *et al.* (2010b) in the fat-tailed dunnart no sexual dimorphism was observed in the three remaining anterior orbital glands, although some males possessed large glands. In the histological examination, the SGTE in the red kangaroo is a multilobar tubuloacinar gland with numerous plasma cells. Curiously, in contrast to the LG, the interlobar septae in SGTE contained numerous single adipocytes. A similar finding was made in the alpaca (KLEĆKOWSKA-NAWROT *et al.* 2015a), Lori sheep (ABBASI *et al.* 2014) and the European bison, in which the adipo-

cytes formed small or large aggregations (KLEĆKOWSKA-NAWROT *et al.* 2015b). The SGTE acini in the red kangaroo were composed of tall conical secretory cells with an eosinophilic cytoplasm. Similar results were also reported by KLEĆKOWSKA-NAWROT *et al.* (2013) in roe deer and KLEĆKOWSKA-NAWROT *et al.* (2015a) in the alpaca, and by PINARD *et al.* (2003) in cattle. Our study found numerous goblet cells in the main excretory duct of the SGTE in the red kangaroo. These cells were not found in the LG. Our study showed that the secretion of SGTE in the red kangaroo had a mucoserous character. Similar secretions were observed in the dog (CAZACU 2010), and domestic and wild ruminants (KLEĆKOWSKA-NAWROT *et al*. 2013; KLEĆKOWSKA-NAWROT *et al.* 2015a; KLEĆKOWSKA-NAWROT *et al*. 2015b; PINARD *et al*. 2003), while REHOREK *et al.* (2010b) showed that the cells of the three non-conjunctival glandular units in the fat-tailed dunnart had mainly serous granules with sparse intracellular lipid droplets.

Histometrical analyses indicated there were no significant differences in the thickness of the capsule, the acinar diameters, tubule diameters and the thickness of the interlobar septae between males and females.

The fine structure of the LG and SGTE has been described in many mammals (ALDANA MARCOS *et al.* 2002; EL-FADALY *et al.* 2014; GARGIULO *et al.* 1999; MARTIN *et al.* 1988), but there are no literature reports of the ultrastructural appearance of this gland in red kangaroo. Ultrastructure of the non-conjunctival glandular units was described by REHOREK *et al*. (2010b) in the fat-tailed dunnart. The fine structure investigations revealed that the LG and SGTE in red kangaroo cells contained drop-like secretory granules of different sizes and low electron-dense secretory granules. Numerous round secretory granules that had moderate to high electron density content were observed. Our study showed that the lipid droplets were more numerous in the LD than in the SGTE. Numerous lipid droplets were also observed in the South American armadillo by ALDANA MARCOS *et al.* (2002), in the dog by MARTIN *et al.* (1988), in the European bison by KLEĆKOWSKA-NAWROT *et al.* (2015b) and in senile rats by EL-FADALY *et al.* (2014). According to REHOREK *et al.* (2010b) the anterior orbital gland in fat-tailed dunnart males occasionally had lipoidal granules. In the red kangaroo, two types of secretory vesicles (Vt1 and Vt2) were also found in the acinar cytoplasm of the LG and SGTE and one type of secretory vesicle (Vt2) was observed in the tubules. Interestingly, type SGTE I vesicles were larger than those in the LG. However, together they were less numerous than the Vt2. KLEĆKOWSKA-NAWROT *et al*. (2015b) found both types of secretory vesicles in the European bison, and they occurred in the LG and SGTE. Similarly, EL-FADALY *et al*. (2014) and ALDANA MARCOS *et al.* (2002) demonstrated the presence of high electron-dense and lower electron-dense secretory granules in acinar cells of the LG in albino rats and South American armadillo. REHOREK *et al.* (2010b) showed,two distinct secretory granules in cells of the three non-conjunctival glands in the fat-tailed dunnart. In females, in the deep glandular units numerous electron-dense granules populated the cell, while in males more electron-lucent granules were present and these cells were typical serous cells with a basal nuclear region and abundant rER. Our study also showed the rER to be more developed in the SGTE of the acinar cells than the LG. Furthermore, large and numerous mitochondria were found in the SGTE acinar cells, but they were small in the LG mitochondria. However, according to MARTIN *et al.* (1988), the rough endoplasmic reticulum was abundant in the LG acinar cells and moderate numbers of rod-shaped mitochondria were observed.

Conclusions

The examinations of accessory organs of the eye in red kangaroo (*Macropus rufus*) revealed the absence of the deep gland of the third eyelid (Harderian gland) in red kangaroo, while in other marsupials from Dasyuridae family the non-conjunctival deep glandular unit (REHOREK *et al.* (2010b) was present. Morphometric study of examined accessory organs of the eye showed that they tended to be larger in males in comparison to females, additionally the lacrimal gland was relatively larger than the superficial gland of the third eyelid. Histological study of upper and lower eyelids showed the presence of organized lymphoid follicles only in the lower eyelid. Diffuse lymphoid tissue in both eyelids was absent. Histological and histochemical study revealed that the lacrimal gland and superficial gland of the third eyelid had a multilobar tubuloacinar structure with numerous plasma cells (especially in LG) and both glands had were mucoserous in character.

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