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HISTOLOGICAL, HISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES ON HARDERIAN AND LACRIMAL GLANDS OF THE CAPERCAILLIE (*TETRAO UROGALLUS MAJOR* L.)

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This study describes the macroscopic anatomy and the microscopic and ultrastructural features of the Harderian gland and lacrimal gland of the Capercaillies. It was conducted both on adult male and female Capercaillies. Tissue sections were stained with hematoxylin and eosin, azan trichrome, modified Mallory's trichrome, methyl green-pyronin Y, periodic acid-Schiff, alcian blue pH 2.5, aldehyde fuchsin and Hale's dialysed iron. The morphometric study of the Harderian and lacrimal glands indicated that they are both larger in male than in female Capercaillies. The histological analysis showed that the HG has a multilobar tubulo-alveolar structure with numerous lymphocytes and plasma cells. The LG has a multilobar tubulo-acinar structure without lymphocytes and plasma cells. The periodic acid-Schiff staining and alcian blue pH 2.5 staining demonstrated a mild positive reaction in the epithelial cells of the Harderian gland and weak positive reaction in the lacrimal gland. The HDI staining detected the presence of carboxylated acid mucopolysaccharides in the Harderian and lacrimal glands. Transmission electron microscopy revealed the presence of two types of secretory vesicles in the cytoplasm of both studied glands. It also showed that lipid droplets and glycogen granules were more abundant in the Harderian gland than in the lacrimal gland of this species.

Keywords: Capercaillie – Harderian gland – lacrimal gland – histochemistry – ultrastructure

INTRODUCTION

The Harderian gland (HG) and lacrimal gland (LG) are the main orbital glands of the lacrimal apparatus [18, 39]. In birds, both glands are considered as a part of the head-associated lymphatic tissue (HALT) [14, 33, 44]. The HG is a dominant orbital gland, located ventromedially to the eyeball near the inter-orbital septum [9]. Based on experiments in thirty-two bird species, Burns [6, 9] classified HGs into three types according to their glandular structure [9]. Type 1 has a compound tubulo-alveolar structure with a lobule composed of one type of epithelial cell with a large age-dependent population of plasma cells, whereas type 2 has a lobule with two types of

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epithelial cells lining the tubule and a much smaller population of plasma cells [9, 38, 44]. Type 3 may be regarded as a "mixed" form [9, 44]. The HG performs a variety of functions including lubrication of the cornea and the third eyelid, production of pheromones and synthesis of growth factors [35, 38, 41, 42, 43]. It is also a site of immune response, particularly in birds [3, 34, 36].

The avian LG is smaller and less developed than the HG [7]. It is located in the dorso-temporal part of the orbit and secretes fluid into the conjunctival space via one or multiple excretory ducts [20, 24]. LG excretory products are part of the tear film which is responsible for moistening, nourishing and lubricating the anterior surface of the eye [10, 46]. The tear film protects the anterior surface of the cornea as well as the superior and inferior conjunctival sacs, allowing gas exchange between the air and the epithelium [18, 46]. The secretory function of the LG together with tear film composition (mucous, aqueous and lipid layers) are important for the ophthalmic physiology and pathology [15, 19].

The aim of the present study was to demonstrate the histological and histochemical structure as well as the ultrastructure of the Harderian and lacrimal glands in male and female Capercaillies. The Capercaillie (*Tetrao urogallus major* L.) is a protected, critically endangered bird, listed on the IUCN Red List of Threatened Species [17]. The results of our research will also be compared with previous studies on other avian species. Detailed knowledge of the morphology of both glands is required for the diagnosis and treatment of ocular diseases in Capercaillie.

MATERIALS AND METHODS

The study was conducted on three adult male (4.35 ± 0.28 kg of body weight) and four adult female (1.61 ± 0.04 kg of body weight) Capercaillies (*Tetrao urogallus major* L.). The Wisla Forest District in Poland (that breeds the birds) provided the study material. The birds used in this study died under natural circumstances. According to the Polish law, the *post mortem* use of animal tissues derived from animals which died of natural causes does not require approval of the Ethics Committee (Act of Animal Protection passed on August 21, 1997 by the Parliament of the Republic of Poland; No. 111/ 724). Immediately after the animals' death, the HG and LG were dissected and measured. The macroscopic studies were conducted using a Zeiss Stemi 2000-C stereomicroscope (Carl Zeiss, Jena, Germany). Morphometric measurements of the glands were conducted using an electronic slide caliber with an accuracy of 0.1 mm. Data were statistically processed with the use of statistical software (Microsoft Office Professional Plus 2010, Microsoft Corporation, Redmond, WA, USA). After fixation in 4% buffered formaldehyde, the HG and LG samples were rinsed in distilled water for 24 h, The material was then processed in an ETP vacuum tissue processor – (RVG3, INTELSINT, Italy), embedded in paraffin and cut into 3–4 μm sections using the Slide 2003 sliding microtome (Pfm medical, Germany). Then, samples were stained with hematoxylin and eosin (H&E), modified Mallory's trichrome and Azan trichrome. In order to perform a histochemical analysis, the follow-

ing stains were used: methyl green-pyronin Y (MGP Y), the periodic acid-Schiff (PAS, pH 2.5), alcian blue (AB pH 2.5), aldehyde fuchsin (AF) and Hale's dialysed iron (HDI) [22, 45]. The histochemical analysis of these glands was conducted in order to identify the presence of neutral or weakly acidic glycoproteins and glycogen, which was stained with a periodic acid-Schiff staining (PAS); sulfated acidic or sialylated mucosubstances which stained with pH 2.5 alcian blue (AB pH 2.5); sulfated acid mucosubstances (SAM) and carboxylated acid mucosubstances (CAM) which was stained with Hale's dialysed iron staining (HDI). Aldehyde fuchsin (AF) was used to detect sulfated acid mucosubstances and elastic fibers. All stainings were carried out in both glands, and the slides were examined under a Zeiss Axio Scope A1 light microscope (Carl Zeiss, Jena, Germany). The histochemical staining scoring system used in PAS, AB pH 2.5, AF and HDI stainings was based on the standard protocol previously described, where (−) indicated a negative reaction; (+) a weak reaction; (++) a mild reaction and (+++) a strong reaction [45].

For the TEM study, the collected samples were fixed in 2.5% glutaraldehyde diluted in 0.1 M phosphate buffer of pH 7.4 and rinsed in a phosphate buffer. The samples were then post-fixed in 4% OsO_4 for 2 h. After rinsing them in phosphate buffer, small parts of the glands were dehydrated in graded acetone series (from 30 to 100%). The dehydrated material was embedded in Epon 812 epoxide resin. The blocks were cut into 70 nm sections using a Leica Reichert Ultra Cut microtome (Leica Microsystem Wetzlar GmbH, Germany). Ultrathin sections were analysed with a Zeiss EM 900 transmission electron microscope (TEM) (Carl Zeiss, Jena, Germany).

RESULTS

Macroscopic and histological observation of HG

The HG of the Capercaillies was located ventromedially, near the inter-orbital septum of the orbit, between the medial straight muscle, pyramidal third eyelid muscle and ventral oblique muscle. The gland had one efferent duct reaching the lower conjunctival sac (Fig. 1a, b). The HG was flattened and elongated and was light pink in colour (Fig. 1b). The mean size (length × width × thickness with SD) of the HG was 12.17 (±0.63) × 5.64 (±0.21) × 1.73 (±0.05) mm in males and 10.21 (±0.54) × 4.08 (±0.16) × 1.52 (±0.04) mm in females (Fig. 1d). The histological study revealed that the HG had a multilobar, branched tubulo-alveolar structure and was surrounded by a thin connective tissue capsule which contained fibroblasts, collagen, elastic fibers and blood vessels. The septa from the capsule penetrated the gland dividing it into elongated lobes of varying size (Fig. 2e). In the center of each lobe, there was a wide lumen of the primary ducts, which was lined by columnar epithelial cells of different height (Fig. 2a). The secondary and tertiary ducts, with a large and irregular lumen, were lined with a layer of cuboidal cells (Fig. 2c). The glandular alveoli were located at the periphery of the lobes and connected short tertiary ducts to wider secondary and primary ducts (Fig. 2c). The alveoli, surrounded by myoepithelial cells, were com-

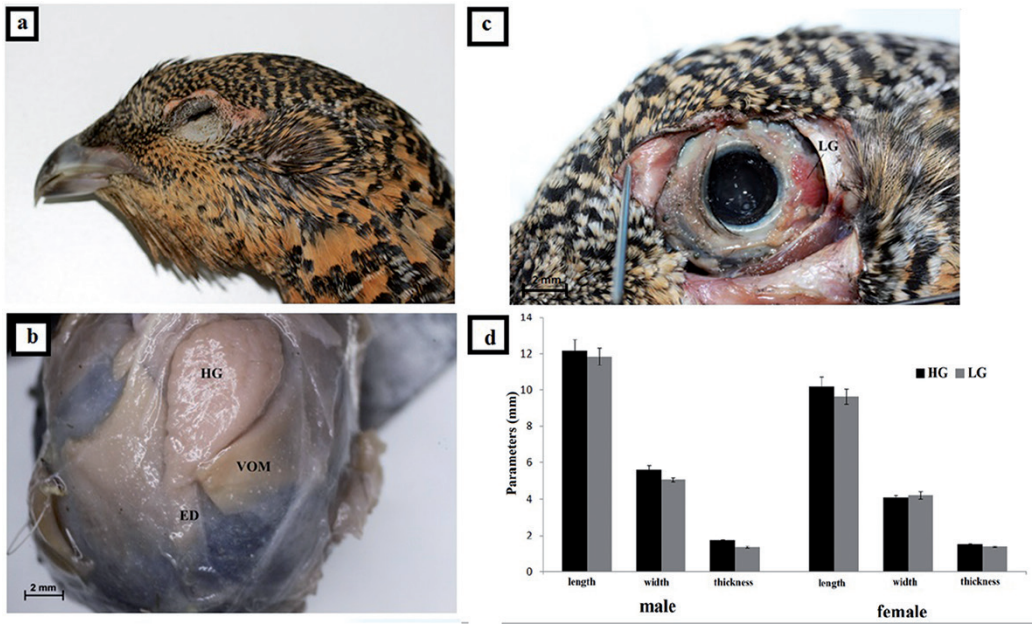
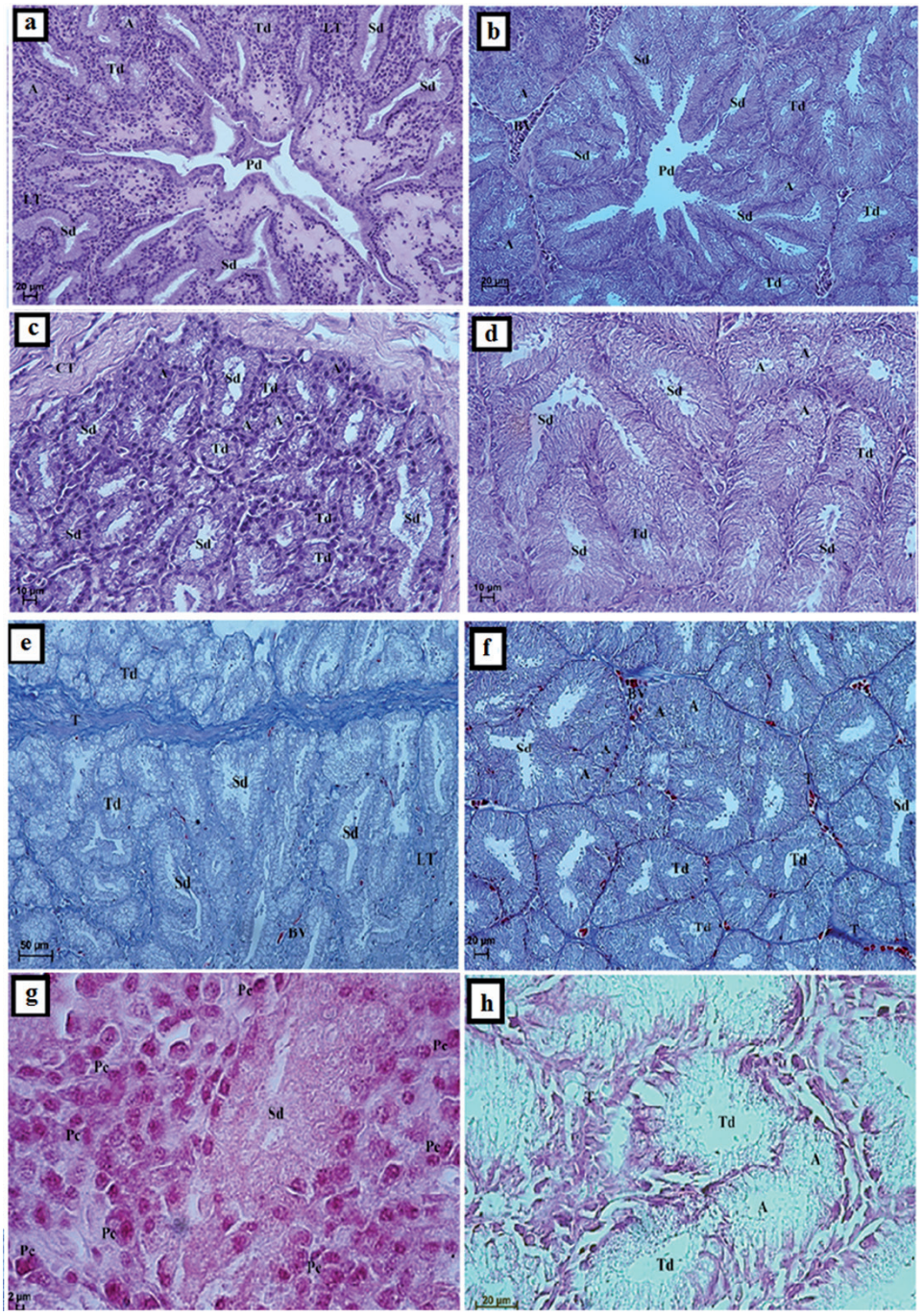


Fig. 1. Macrograph of the HG (Harderian gland) and LG (lacrimal gland) in Capercaillie. **a)** The area of the Capercaillie head; **b)** HG, Bar = 2 mm; **c)** LG, Bar = 2 mm; **d)** Histometric parameters of the HG and LG in male (n = 3) and female (n = 4) of Capercaillie. Values are given as mean ± standard error. VOM – ventral oblique muscle, ED – efferent duct

posed of pyramidal cells with a slightly granular basophilic and vacuolated cytoplasm. The oval nuclei of the alveoli cells were located at the basal portion of the cells (Fig. 2c). The peripheral lobular interstitium, interalveolar interstitium, and interstitium between the secondary and tertiary ducts of the HG were filled with numerous lymphocytes and plasma cells (Fig. 2g). Some lymphatic cells also surrounded the primary ducts (Fig. 2a).

Fig. 2. Light micrograph of the HG (a, c, e, g) and LG (b, d, f, h) in Capercaillie. **a**) Note numerous lymphatic tissue between tertiary and secondary ducts and single primary duct, H&E staining, Bar = 20 µm; **b**) Numerous visible blood vessels and primary duct, H&E staining, Bar = 20 µm; **c**) Visible alveoli, tertiary and secondary ducts, H&E staining, Bar = 20 µm; **d**) Visible acini, tertiary and secondary ducts, H&E staining, Bar = 20 µm; **e**) Note the collagen fibers of interlobares septa and mucous secretion in glandular cells (blue colour), Mallory trichrome staining, Bar = 50 µm; **f**) Note the collagen fibers of interlobares septa and mucous secretion in glandular cells (blue colour), Azan trichrome staining, Bar = 20 µm; **g**) Note the numerous plasma cells, MGP Y staining, Bar = 2 µm; **h**) Absence of plasma cells, MGP Y staining, Bar = 20 µm. C – capsula, T – trabecule, CT – connective tissue, L – lobus, A – alveoli/acini, Pd – primary ducts, Sd – secondary ducts, Td – tertiary ducts, LT – lymphatic tissue, BV – blood vessels, Pc – plasma cells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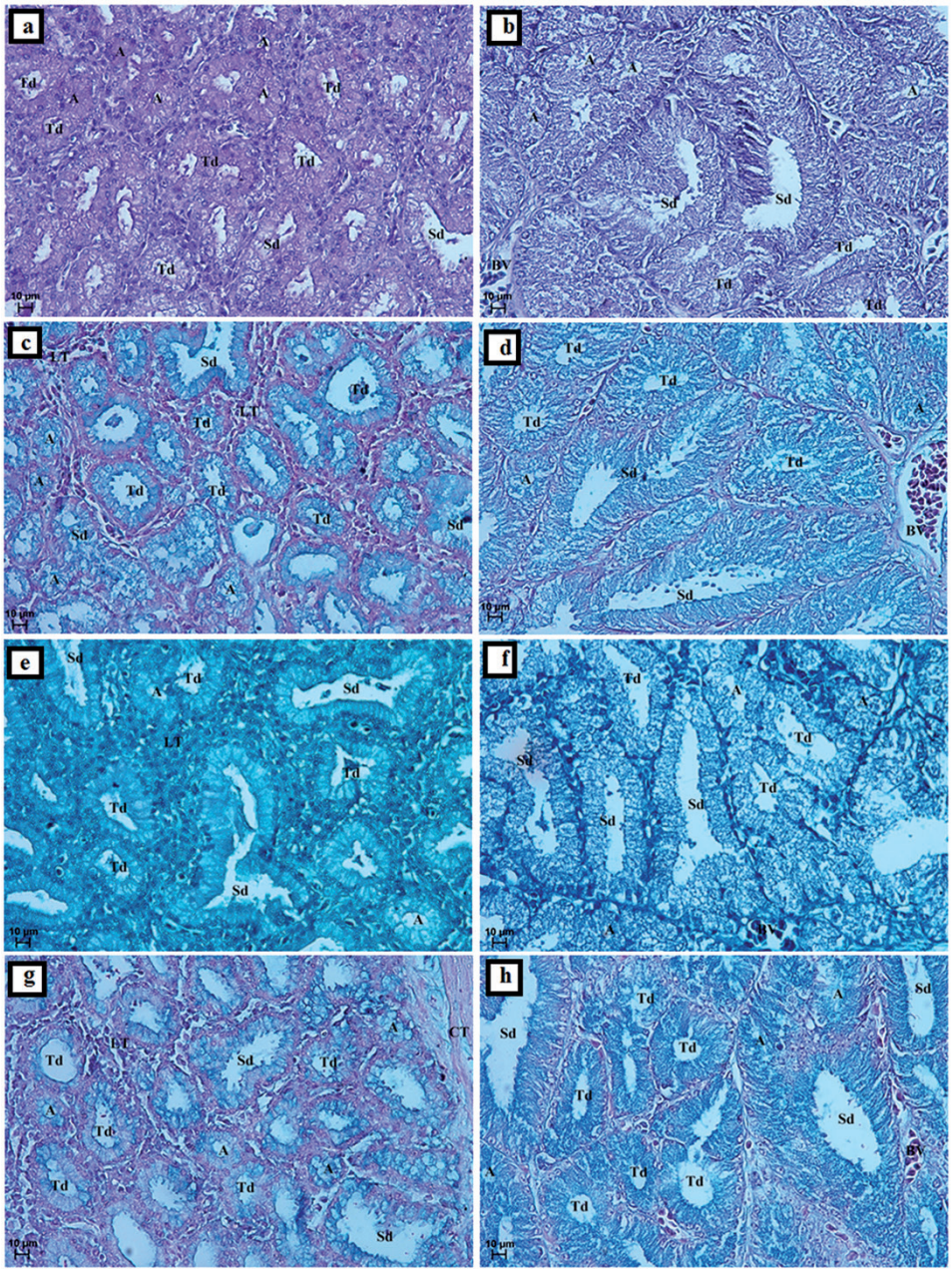
Macroscopic and histological observation of LG

The LG was placed dorsolaterally between the lateral straight and dorsal straight muscles, close to the pyramidal third eyelid muscle and tendon. The gland was uniform, undivided, flattened in shape and bright red in colour (Fig. 1a, c). The LG secretes fluid via multiple ducts, which opened into the conjunctival space beneath the lower eyelid (Fig. 1c). The mean size (length × width × thickness) of the LG was 11.85 (±0.47) ×5.06 (±0.1) ×1.36 (±0.04) mm in males and 9.62 (±0.42) × 4.22 (±0.2) × 1.38 (±0.04) mm in females (Fig. 1d). The LG was covered by a thin capsule of connective tissue, with septa dividing the gland into lobes of varying size. Those lobes were then subdivided into lobules. The connective tissue was composed of fibrocytes, collagenous fibers, adipose tissue and a large amount of blood vessels (Fig. 2b, f). The LG had a multilobar, tubulo-acinar structure. The acini were surrounded by myoepithelial cells. Each acinus was composed of tall conical cells, which formed a small lumen. The nuclei of the acinar cells were ovoid in shape and were located in the basal portion of the cytoplasm. These cells had a granular basophilic and vacuolated cytoplasm. Glandular secretion formed aggregates within the whole cell (Fig. 2f). A wide lumen of the primary duct was located in the central part of the each lobe. The primary ducts were lined with columnar epithelial cells of varying heights (Fig. 2b). The secondary and tertiary ducts were lined with cuboidal cells. The nuclei of these cells were oval and were located in the basal portion of the cells (Fig. 2d). Lymphocytes and plasma cells were not found in the peripheral lobular interstitium, interacinar interstitium, and interstitium between tertiary and secondary ducts of the LG (Fig. 2h).

Histochemical examination of HG

MGP Y staining confirmed the presence of numerous lymphocytes located between HG plasma cells. Those plasma cells had a characteristic blue nucleus and pink cytoplasm (Fig. 2g). We observed a mild positive reaction in the alveoli, and tertiary and secondary ducts stained with PAS assessed as (++), that was demonstrated through the presence of blue nuclei and a pink cytoplasm in the glandular cells (Fig. 3a). The staining with (AB pH 2.5) detected the presence of a mildly positive reaction (++) in the alveoli and tubules (Fig. 3c). The AF staining showed a negative reaction (–) in

Fig. 3. Light micrograph of the HG (a, c, e, g) and LG (b, d, f, h) in Capercaillie. **a**) Visible middle positive reaction (++) in alveoli and tertiary and secondary ducts, PAS staining, Bar = 10 μm; **b**) Visible weakly positive granules (+) in the glandular acini and tertiary and secondary ducts, PAS staining, Bar = 10 μm; **c**) Visible middle positive reaction (++) in alveoli and tubules, AB pH 2.5 staining, Bar = 10 μm; **d**) Visible weakly positive granules (+) in the glandular acini and ducts, AB pH 2.5 staining, Bar = 10 μm; **e**) Visible negative reaction (–) in alveoli and ducts, AF staining, Bar = 10 μm; **f)** Visible negative reaction (–) in acini and ducts, AF staining, Bar = 10 μm; **g**) Visible weakly positive reaction (+) in alveoli, tertiary and secondary ducts, HDI staining, Bar = 10 μm; **h**) Visible middle positive reaction (++) in the acini, tertiary and secondary ducts, HDI staining, Bar = 10 μm. CT – connective tissue, A – alveoli/acini, Sd – secondary ducts, Td – tertiary ducts, LT – lymphatic tissue, BV – blood vessels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the alveoli and ducts (Fig. 3e). The HDI staining indicated a weakly positive reaction (+) in the alveoli as well as in tertiary and secondary ducts, which pointed to the presence of carboxylated acid mucopolysaccharides (Fig. 3g).

Histochemical examination on LG

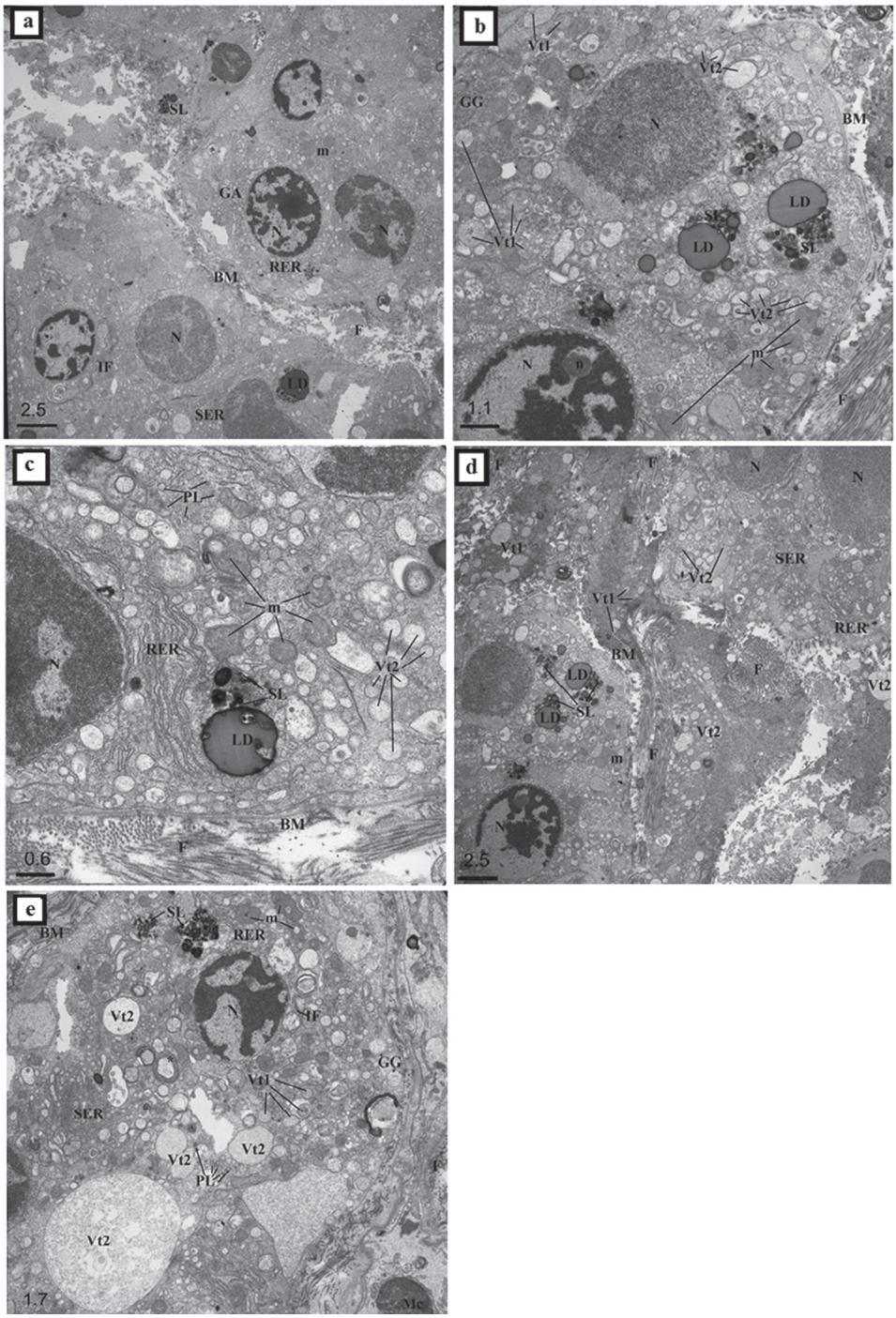
The PAS staining of the LG demonstrated the presence of secretory cells containing weakly positive granules (+) in the glandular acini and tertiary and secondary ducts (Fig. 3b). Staining which used the pH 2.5 AB method demonstrated the presence of weakly positive granules (+) located in the glandular acini and ducts (Fig. 3d). The AF staining showed a negative reaction (–) in the acini and ducts (Fig. 3f). The HDI staining showed a mildly positive reaction (++) in the acini and tertiary and secondary ducts, which indicated the presence of blue carboxylated acid mucopolysaccharides (Fig. 3h).

There was no evident histological and histochemical difference in the studies between the male and female Capercaillies.

TEM studies

The secretory HG and LG cells in Capercaillies had a similar ultrastructural appearance. Both glands had a tubulo-alveolar (HG)/tubulo-acinar (LG) structure and epithelial alveoli/acini with duct cells and a basally located circular nucleus of a uniform chromatin pattern and a single nucleolus. The nuclear envelope was composed of two distinct membranes around the perinuclear cisterna and was clearly visible (Fig. 4b, e). The interlamellar spaces were frequently dilated and contained a homogeneous, moderately dense material. The plasma cells had an ovoid, basally located nucleus with condensed heterochromatin arranged in clumps near the nuclear membrane (Fig. 4c). Plasma cells were absent in the LG. Numerous small primary lysosomes and sparse glycogen granules were present in the cytoplasm of the HG and LG (Fig. 4b, e). Numerous, large oval mitochondria with frequently observed transverse cristae and a dense matrix were observed in the cytoplasm of both HG and LG (Fig. 4a, e). The mitochondria were associated with cisternae of rough endoplasmic reticulum (RER) (Fig. 4a, e). In the secretory cells, large lipid droplets were in close association to with secondary lysosomes, which had a weakly visible membrane with

Fig. 4. Survey electron micrograph of the HG (a, b, c) and LG (d, e) cells in Capercaillie. **a**) Note the secretory cells, Bar = 2.5 µm; **b)** Note secretory cells with vesicle types 1 and 2, Bar = 1.1 µm; **c**) Visible part of plasma cell, Bar = 0.6 µm; **d)**. Note the secretory cells, Bar = 2.5 µm; **e)** Note secretory cells with vesicles types 1 and 2, Bar = 1.7 µm. N – nucleus, n – nucleolus, RER – rough endoplasmic reticulum, SER – smooth endoplasmic reticulum, FR – free rybosomes, m – mitochondria, GA – Golgi apparatus, PL – primary lysosomes, SL – secondary lysosomes, GG – glycogen granules, LD – lipid drops, F – filaments, IF – intermediate filaments, * showing the presence of extensive membrane structures arranged in concentric lamellae, Vt1 – vesicles type 1, Vt2 – vesicle type 2, BM – basement membrane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electron-dense small oval lipids (Fig. 4a, b, d). The lipid droplets and the secondary lysosomes were much more numerous in the HG than in the LG. The GERL system was observed within the HG and LG, which was formed by the Golgi apparatus, smooth endoplasmic reticulum (SER) and lysosomes. The Golgi apparatus located close to the nucleus and was composed of a low and flat cistern layer and narrow vesicles and vacuoles (Fig. 4a). The HG and LG had electron-dense granules situated in the peripheral region of each cell or near the nucleus. Two types of vesicles were observed in the HG and LG cytoplasm. Numerous oval granules of various sizes with a moderate to high electron density (type 1) were seen in the cytoplasm (Fig. 4b, e). The cells with granules of various sizes with a much lower electron density (type 2) were identified in both glands (Fig. 4b, e). They were positioned very close to the mitochondria and RER. Within the intracellular space, numerous filaments were visible (Fig. 4a, d). Myoepithelial cells in the HG and LG were fusiform and located all over the basal surface of the secretory cells (Fig. 4e). Myoepithelial cells contained elongated nuclei and characteristic bundles of microfilaments, cytoplasmic dense bodies and caveolae. No evident ultrastructural difference between the glauds of the male and female Capercaillies could be observed.

DISCUSSION

The Capercaillie is the largest member of the grouse family – a group of birds from the *Galliforms* order, in the *Phasianidae* family, which are characterized by pronounced sexual dimorphism. Males are significantly larger than females. That was also demonstrated in the examined birds, both in terms of their weight and the size of their examined glands. The Capercaillies are territorial defenders, and exhibit aggressive behaviour. Pheromones, believed to be produced by the Harderian gland, are likely to play an important role in the behaviour of these birds, as they do in rodents [43].

In birds, numerous studies concentrated on the HG [2, 29, 31] and just a few on the LG [7, 10, 14]. In our study, the HG of the Capercaillies was located ventromedially, near the inter-orbital septum of the orbit, between the medial straight muscle, pyramidal third eyelid muscle and ventral oblique muscle. This location was similar to that of other avian species [2, 7, 30, 44]. In the examined birds, the HG was flattened and elongated in shape and light pink in colour. Boydak and Aydin [4], and Wight et al. [47] reported that the gland was elongated, pale pink to brownish in colour and strap-like in shape in domestic geese and fowl. Burns [9] reported that the HG in was almost hemispherical in ducks and pelicans and triangular in the rockhopper penguin. The LG of the Capercaillies was placed dorsolaterally between the lateral straight and dorsal straight muscles, close to the pyramidal third eyelid muscle and its tendon. The location of the gland was similar in other other avian species, e.g. turkey, chicken and duck [7, 11]. In Capercaillies, the LG was uniform, undivided, flattened in shape and bright red in colour. According to Klećkowska-Nawrot et al. [28], the LG in Bilgorajska geese was bright red in colour and had a triangular shape. On the other hand, the gland was oval in shape and of bright red colour in the African black ostrich [26].

The averaged results indicated that HG in Capercaillies was relatively larger than the LG. The differences in the size of HG were also observed in Japanese quails, chickens, the common pheasant and domestic fowl [9, 14, 32, 47]. The morphometric study of the HG in Capercaillie also indicates that this gland is larger in males than females. One of the characteristics of the HG is sexual dimorphism, which was found in the rodents like the golden hamster, with structural differences between male and female HGs [5, 12, 43]. These differences are most likely functional. Similar results were obtained when analyzing the size of the LG. However, no evident difference between the male and female glands was observed in histological, histochemical and ultrastructural studies.

In the histological examination, the HG in the Capercaillie was defined as a type 1 tubulo-alveolar gland, according to Burns [9], with numerous plasma cells as described in other birds species [4, 9, 25]. Numerous lymphocytes and plasma cells were found in the peripheral lobular interstitium, interstitium between alveoli as well as the tertiary and secondary ducts of the HG. A similar finding was described in broiler and native chickens [21, 23]. Similarly to other studies, the HG of all the birds in this study was infiltrated by plasma cells, although there were also foci of lymphocytes [1]. In contrast, a small amount of plasma cells was found in the osprey [30]. The population of plasma cells can vary between males and females [21]. In the studied Capercaillie, there were no differences in the number of plasma cells between males and females. The large number of plasma cells detected in the studied birds may indicate their exposure to various pathogens and may indicate better resistance. The histological studies revealed that the LG had a multilobular tubulo-acinar structure similar to that of the HG. However, the connective tissue of the LG in the Capercaillie contained adipose tissue, which had not been previously described in other birds, but which is characteristic for some mammals, such as the alpaca [27]. The parenchyma of the examined LG did not contain lymphatic or plasma cells.

The HG and LG of Capercaillies were composed of alveoli/acini and many tertiary and secondary ducts and single primary duct. The alveoli/acini were composed of pyramidal cells with a slightly granular basophilic and vacuolated cytoplasm. Additionally, those secondary and tertiary ducts with a large and irregular lumen, were lined with a layer of cuboidal cells. Similar results were also reported by Altunay and Kozlu [2], Boydak and Aydin [4], Dimitrov and Genchev [12], Dimitrov and Nikiforov [14], Khan et al. [21] and Kozlu et al. [30].

Histochemical studies performed on the HG of Capercaillies revealed a mild PAS-positive reaction in the alveoli and tertiary and secondary ducts. On the other hand, a weakly PAS-positive reaction was found in LG acini and tertiary and secondary ducts, indicating neutral and acid mucins as well as glycoprotein secretions in these glands. Similar results were obtained in the study on ducks [14], turkeys [24, 26], domestic geese [4], fowl [47], young chickens [40] and African black ostriches [25, 26]. The AB pH 2.5 method confirmed the presence of acid mucins in the Capercaillie HG and LG. The AB-positive cells were observed in both the glandular and ductal epithelium. AB-positive cells were also observed both in the corpus of the glandule and within the main duct in HG and LG in chickens [32], domestic ducks [5], domes-

tic geese [4], Bilgorajska geese [28], fowl [8] and ospreys [30]. In the present study, the HDI staining showed the presence of carboxylated acid mucopolysaccharides in the HG and LG. A similar reaction, revealing blue granules that indicated the presence of carboxylated acid mucopolysaccharides was observed in fowl, turkeys and ducks [8].

Fine structural investigations revealed that the HG and LG cells contained drop-like secretory granules of different sizes, large lipid vacuoles and low electron-dense secretory granules. Numerous round secretory granules with content of moderate to high electron density were also observed. In the HG of domestic fowl, Rothwell et al. [40] described the presence of four types of secretory cells, with a different structure of cell organelles. These differences were connected with the appearance of the nucleus, mitochondria, Golgi apparatus, rough endoplasmic reticulum, and the amount as well as the size of the secretory vesicles (type 1 and type 2). According to Rothwell et al. [40] type 1 and type 2 secretory cells were dominant in young birds. However, types 3 and 4 were more prominent in old birds. Our study showed that the secretory cells in the HG were characterized as type 3. Type 3 secretory cells contained a stack of rough endoplasmic reticular lamellae, elongated, branched and dense mitochondria and a very prominent Golgi apparatus as well as numerous large secretory vesicles. Maxwell et al. [31] found that secretory cells in the turkey HG to had mitochondria with moderately dense matrixes. Additionally, these authors also found dense osmiophilic rods or crystalline structures, which was not evident in our study. Crystalline inclusions within the rough endoplasmic reticulum have been widely documented [16]. In this study, the HG had large lipid vacuoles, which were close to secondary lysosomes, while single vacuoles were present within the LG. Similar results were obtained by Wight et al. [47] in the HG of domestic fowl, which is in accordance with the histochemical finding that the gland is mucous-secreting. According to Maxwell et al. [31], there are secretions in the HG cells of mucous-containing vesicles and basal aggregations of non-secreting lipid-like droplets. Paule and Hayes [37] demonstrated that the mucous nature of secretory cells is characteristic for birds, in contrast to mammals that secrete lipid. In reptiles, in turn, the secretions are serous or seromucous. The examined LG cells had a large number of secretory granules, which varied in size. They were localized in the peripheral cell compartments, which corresponded with the histochemical results, indicating the mucous secretion of the gland. The majority of the secretory cells contained secretory granules, predominantly of a moderate to high electron density (type 1).

In conclusion, the Harderian and lacrimal gland in Capercaillies have similar histological and fine structures in comparison to other avian species. The composition of this glandular secretion is also similar. Histochemical studies demonstrated that the Harderian and lacrimal glands mainly contain acid mucopolysaccharides. This finding suggests that the secretory role of the Harderian and lacrimal glands in the production of tear film is similar. Immune cells (mostly plasma cells) were observed only in the Harderian gland, which confirms their role in immunological reactions of the head-associated lymphatic tissue (HALT).

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