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RESEARCH ARTICLE

MACRO- AND MICROANATOMICAL STUDY OF THE ORBITAL GLANDS OF THE COMMON MOORHEN "*GALLINULA CHLOROPUS*"

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ABSTRACT

Numerous and variable studies have been done about the avian orbital glands. However, more comparative investigations including several species need more attention. The current study intends to investigate the relationship between the morphological, histological, and histochemical features of the orbital glands of the common moorhen (*Gallinula chloropus*), and their protective function for the eye. There are two lacrimal glands in the common moorhen, which differ in size, shape, location, and position of their openings. The dorsal lacrimal gland appears as a bilobed cylindrical gland with dorsoventrally compressive tissues situated within the fossa in the dorsal orbital rim, and unites anteriorly beneath the lacrimal bone to form its draining duct. A small lateral lacrimal gland is located on the posterior pole of the eyeball. The Harderian gland appears voluminous with an irregular, multi-lobed shape. Histologically, the orbital glands are composed of compound tubulo-alveolar type, with each acini lined with cuboidal cells with large spherical nuclei and covered with a pigmented capsule. These glands reveal various positive reactions with periodic acids Schiff's reagent and Alcian blue (pH = 2.5). The present study concluded that the common moorhen possesses different types of orbital glands: two lacrimal glands and a voluminous Harderian gland. The current study suggested that the orbital glands change their features in this bird that catches their food from underwater to adjust the water-visual refraction; furthermore, according to their acidic secretions, this gland may allow the protection of the eye against any water pollution.

INTRODUCTION

The orbital glands, eyelids, and ocular muscles are among the ocular adnexa of the eye, which are crucial components of the ocular system[1-3]. The major orbital glands in vertebrates are the lacrimal and Harderian glands[4-7]. The anatomical structure of the orbital glands exhibits great variability among bird species[8,9]. The previous studies mentioned the anatomical localization of the avian orbital glands. It has been recorded that the lacrimal gland is located in the periorbital space in the dorso-temporal region of the orbit[5,10]. The Harderian gland is situated medioventrally to the eyeball, adjacent to the interorbital septum between the medial rectus muscle, the pyramidal muscle, and the ventral oblique muscle[7,11-15]. The Harderian gland is present in most terrestrial species that have a nictitating membrane. Among mammalian species, the Harderian gland is documented to be absent in terrestrial carnivores and bats, and it is thought to be rudimentary in monkeys[16]. It was first mentioned by Johann Jacob Harder in 1694[17], and has since been determined to be a para-orbital gland. The Harderian gland appears in some animals as large and more developed compared to the lacrimal gland[12,13,18,19]. Reem and Khattab[5] stated that the Harderian gland operates as another lacrimal gland at the base of the nictitating membrane.

The primary function of the orbital glands is to release mucous, serous, or seromucous fluids as a component of the precorneal film[10]. According to Rothwell *et al.*[3] and Frahmand and Mohammadpour[20], the avian Harderian gland was regarded as a lymphoid organ with abundant populations of lymphocytes and plasma cells in the connective tissue of the stroma, which increase with age. Rothwell *et al.*[3] documented that the Harderian gland of the domestic fowl has a merocrine mode of secretion. Meanwhile, Frahmand and Mohammadpour[20] documented that the lacrimal and Harderian glands of the ostrich both have an apocrine mode of secretion. The main role of the lacrimal gland is the secretion of the tear fluid that moistens and lubricates the anterior ocular surface[21]. The tear fluid provides protection for the front side of the cornea and the two conjunctival fornix, and enables the exchange of gases between the air and the epithelium[21,22]. The tears contain numerous soluble antibacterial agents that protect the ocular surface from infections[23]. Hirayama *et al.*[24] illustrated the ability of bio-engineered organ replacement to functionally restore the lacrimal gland. Clear vision requires a clear smooth, well-lubricated surface, especially for animals that live in arid, hot, and sandy environments[22]. The tear film and the conjunctiva-associated lymphoid tissue contribute to maintaining the health of the cornea[22], play a significant role in the physiology and pathology of the eye[5,25], and also provide nutrients to the eye[10,26-29]. The Harderian gland's functions include lubrication of the cornea and the nictitating membrane and the synthesis of growth factors and pheromones[30]. It is also an immunological site, particularly in birds[7,31,32]. According to Van Ginkel *et al.*[33], the ocular immunization with Ad5-H5 induced antigen-specific, humoral immune responses in the Harderian gland. Baccari *et al.*[34] mentioned that the Harderian gland plays an important role in osmoregulation, and described it as a salt source in some tortoises. Chieffi *et al.*[16] demonstrated its role in thermoregulation in some rodents. Among these are sexual dimorphism, photosensitivity, synthesis of hormones, and assisting thermoregulatory behaviors and production of pheromones[11].

The scientific community has taken a renewed interest in the investigation of how the orbital glands in diverse vertebrates relate to vision and other senses. Therefore, numerous studies have recently investigated the extra-orbital functions of the orbital glands. For instance, Al-Nefeiy *et al*.[4] have clarified how the orbital glands influence vision and olfaction. Rehorek *et al.*[9] have revealed the correlation between these glands and the vomeronasal organ, which is involved in reptilian olfaction. However, knowledge of the typical anatomical and histological features of the orbital glands in some birds is nonetheless insufficient. To understand the functions of both types of orbital glands, numerous anatomical, biochemical, and physiological characteristics are required. With only few publications available, scientific research should devote more attention to both glands in a variety of avian species. Those were the main reasons for the present study. The current study investigated the anatomical, histological, and histochemical features of the orbital glands in the common moorhen, as an aquatic bird.

MATERIAL AND METHODS

The common moorhen, *Gallinula chloropus*, is a distinctive diving bird blackish with a red and yellow beak and long green legs. Ten specimens of adult common moorhen were obtained in January 2019, from Abu-Rawash (Giza, Egypt), where they inhabit around Al-Mansouriya canal and consume a wide variety of aquatic vegetation beside or in the water. The common moorhen has been carefully identified and recorded in the National Center for Biotechnology Information (GenBank/NCBI) under the accession number (OM943961.1). The specimens were brought alive to the lab of comparative anatomy of vertebrates and dissected according to the recommendations of the Assiut University research ethics committee (ethical approval number: 0620230097). Each head was isolated from the body after anesthesia in order to prepare it for the following examinations.

Anatomical investigations

Each head of the bird was initially preserved for three weeks in formalin (10%), and then moved to phenoxy-ethanol (2%). A digital camera (Samsung Galaxy A52, model number: SM-A525F/DS) was used to take the photographs after meticulously dissecting the orbital glands under a Wild Heerbrugg steromicroscope (Switzerland).

Histological and histochemical investigations

Whole eyes and orbital glands of each species were fixed in neutral formalin (10%) for the histological investigation, dehydrated in ethyl alcohol at ascending concentrations, cleared in xylene, embedded in paraffin wax at 58–62°C, and then sectioned into 7 μm slices using a Leica rotatory microtome (RM 20352035; Leica Microsystems, Wetzlar, Germany). Slices were stained in conformity with Bancroft and Gamble staining procedures[35], using Masson's trichrome stain for detecting collagen fibers and periodic acid Schiff's (PAS) reagent and Alcian blue (pH = 2.5) for demonstrating the neutral and acid glycosaminoglycan, respectively.

RESULTS

Description of lacrimal gland

There are three orbital glands in common moorhen, *Gallinula chloropus*: lacrimal gland, which is distinguished into lateral lacrimal gland and dorsal lacrimal one, and Harderian gland. According to the macro-anatomical investigation of the eye of the common moorhen, *Gallinula chloropus*, the lateral and dorsal lacrimal glands were differing in size, shape, and the place where they are opening. The lateral lacrimal gland of the common moorhen, *Gallinula chloropus*, appeared small in size, narrow in thickness, rectangular in shape, and slightly pink in color with dark patches. This gland is situated at the posteriolateral part of the orbit behind the postorbital process and posterior to the lateral canthus of the eye, covered by the periorbital sheet. The lateral lacrimal gland drains its secretions *via* a single wide duct that opens into the conjunctival fornix at the lateral canthus below the lower eyelid. The dorsal lacrimal gland is a bilobed gland that is elongated crescent-shaped and also slightly pink in color. It is housed within the supraorbital fossa of the frontal bone that occupies the distance between the post-orbital and lacrimal process. The dorsal lacrimal gland consists of two large lobes; the external lobe is thinner and longer than the internal one. The two lobes united together anteriorly beneath the lacrimal process emerging a single duct. This duct extends under the lacrimal process passing dorsal to the maxillary bone to open into the nasal cavity (Figures 1 and 2).

Micro-anatomical investigation of the lateral lacrimal gland of the common moorhen exhibits that this gland is externally enclosed by a thin collagenous capsule, which then releases many trabeculae dividing the gland into unorganized lobes of various sizes, and those lobes are further divided into lobules. The connective tissue septa of the lateral lacrimal gland are abundant in myoepithelial cells, collagen

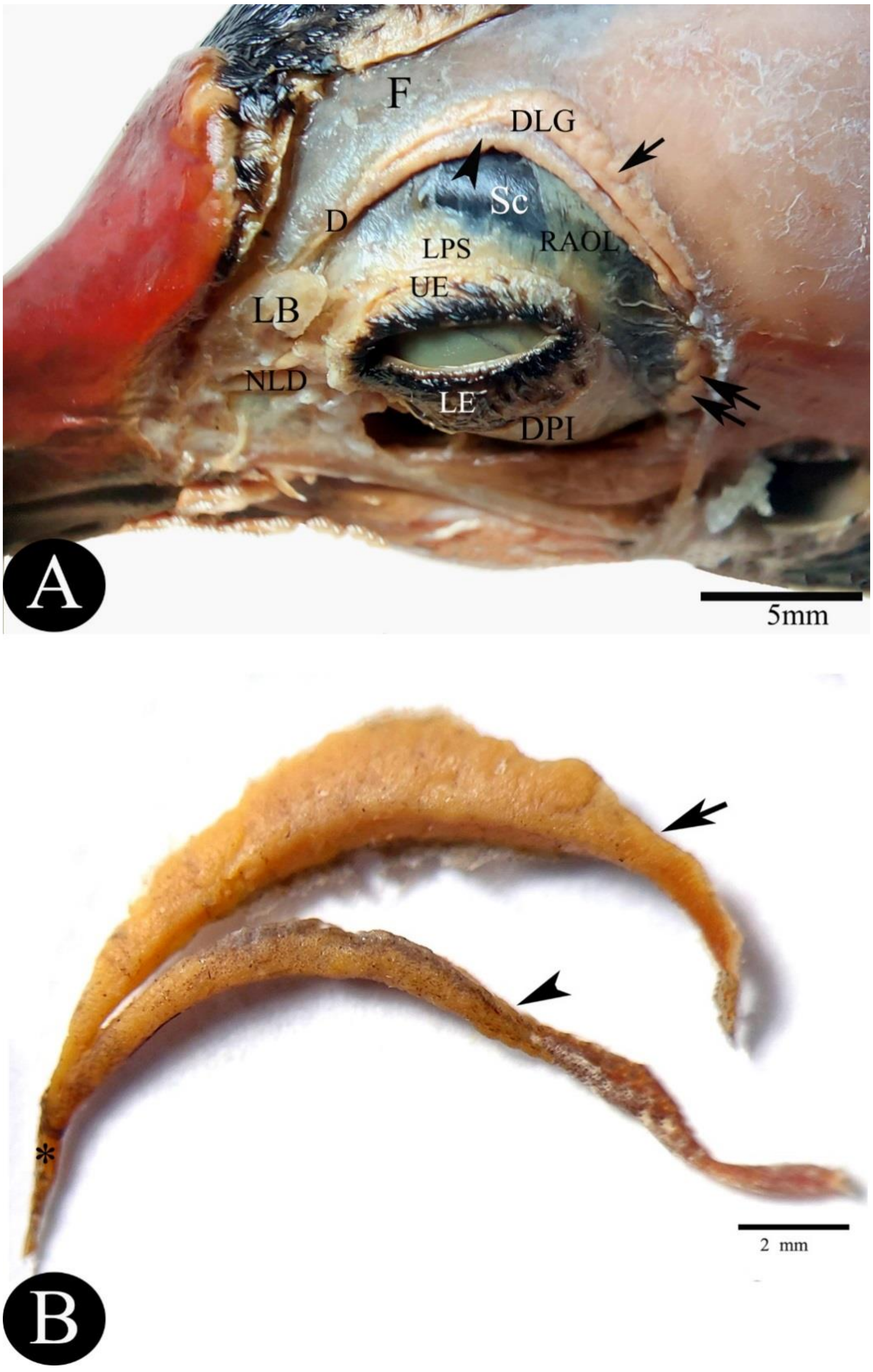

Figure 1: Photomacrograph of the dorsal lacrimal gland in common moorhen, *Gallinula chloropus*, showing **(A)** the head of the common moorhen after removing partially the eyelids and completely the periorbital sheet to show the two lobes of dorsal lacrimal gland (DLG); the dorsal lobe (arrow) and the ventral lobe (arrow head), with its main duct (D), and the lateral lacrimal gland (green arrow). In addition, the lacrimal bone (LB), nasolacrimal duct (NLD), retractor anguli oculi lateralis muscle (RAOL), levator palpebral superioris muscle (LPS), depressor palpebral inferioris muscle (DPI), the sclera (Sc), the upper eyelid (UE), and the lower eyelid (LE) were also shown (scale bar = 5 mm). **(B)** Isolated dorsal lacrimal gland with its the external lobe (arrow), internal lobe (arrow head), and their main duct (star) (scale bar = 2 mm).

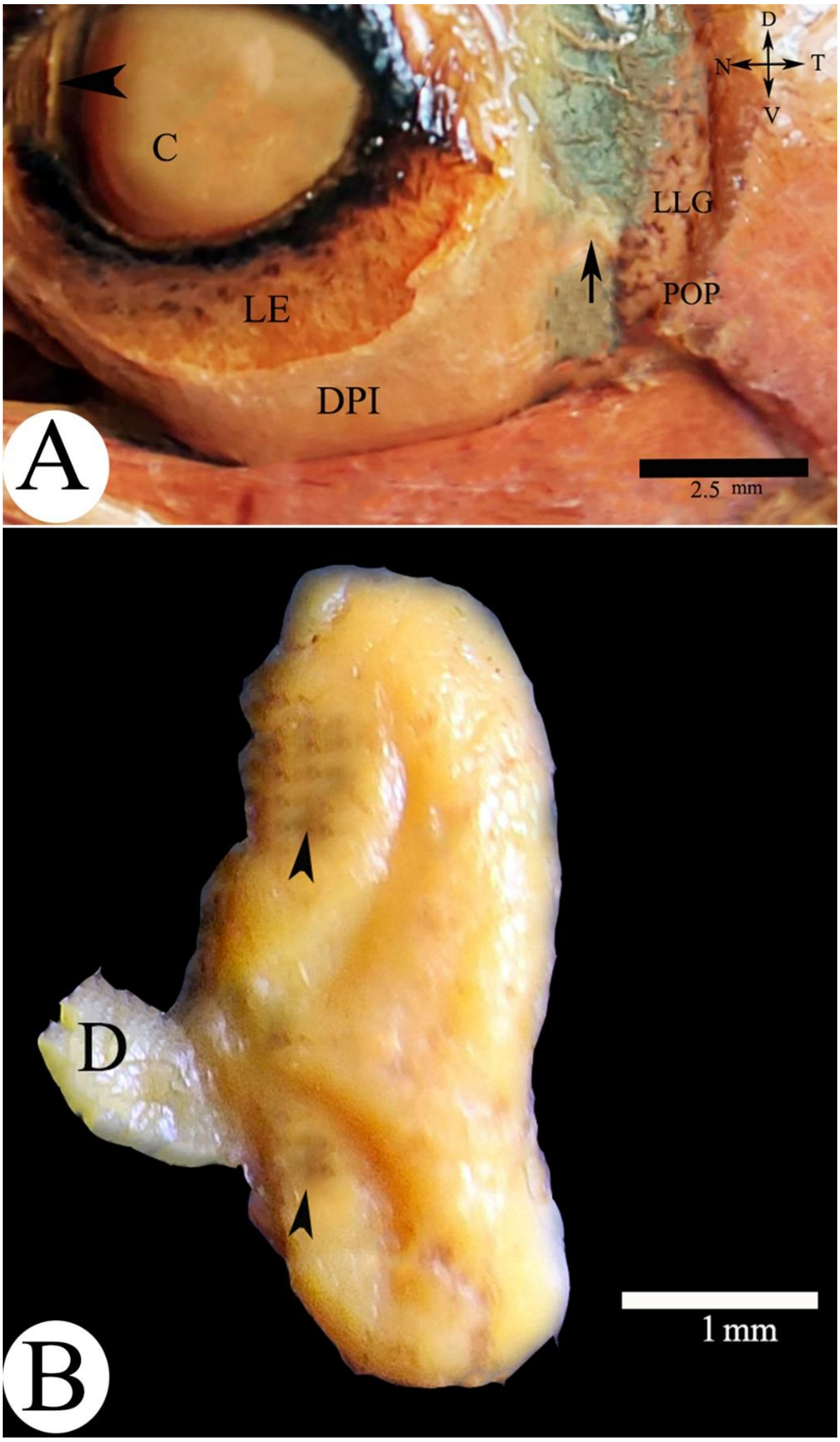

Figure 2: Photomacrograph of the head of common moorhen, *Gallinula chloropus*: **(A)** after removing partially the eyelids and completely the periorbital sheet showing the lateral lacrimal gland (LLG) anterior to the postorbital process (POP) and its duct (arrow), the cornea (C), the lower eyelid (LE), nictitating membrane (arrow head), and the depressor palpebral inferior muscle (DPI) (scale bar = 2.5 mm), **(B)** isolated lateral lacrimal gland with many patches of dark pigmentation (arrow head) and it's draining duct (D) (scale bar = 1 mm).

fibers, and blood vessels despite their thinness. Meanwhile, the dorsal lacrimal gland is enclosed by a very thick sheet of collagenous connective tissue, which sends a thick septa of collagen fibers to separate the two lobes. Each lobe of the dorsal lacrimal gland consists of secretory tubular-acinar units and a central duct, which is lined with stratified cuboidal cells (Figures 3 and 5). Each acini consists of cuboidal cells leaving a small lumen. The acinar cells have rounded nuclei that are located in the basal portion of the cytoplasm. Granular, vacuolated, basophilic cytoplasm fills the acini. The histochemical analysis of the two lacrimal glands demonstrates that the secretory cells have a moderately positive reaction with PAS stain, but exhibit a strongly positive reaction to Alcian-blue (pH = 2.5), which indicates the presence of acid mucin (Figures 4 and 6).

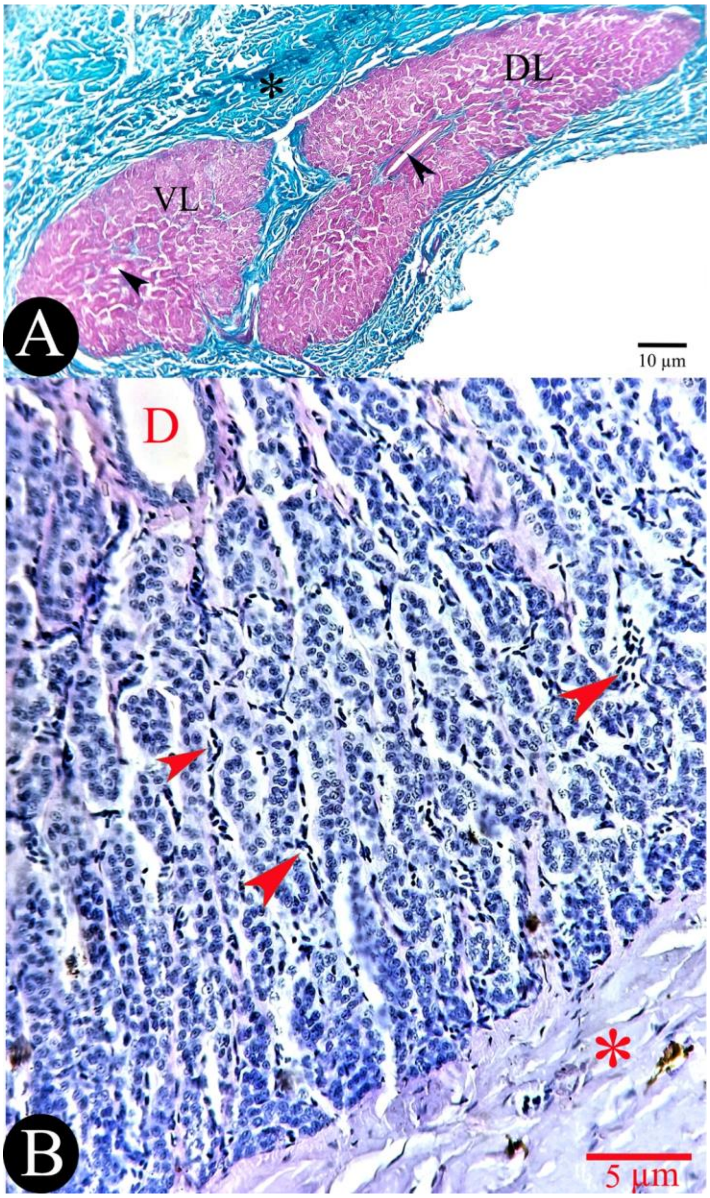

Figure 3: Photomicrograph of a transverse section of the dorsal lacrimal gland of common moorhen, *Gallinula chloropus*, showing: **(A)** the two lobes of this gland; dorsal lobe (DL) and ventral lobe (VL) a thick capsule of collagen fibers (star), and the main duct (D) (Masson's trichromic stain, scale bar = 10 µm), **(B)** the tubulo-alveoli acini lined by columnar cells and surrounded by thick capsule (star), the draining duct (arrow head), and myoepithelial cells (arrow head) (hematoxylin and eosin stain, scale bar = 5 µm).

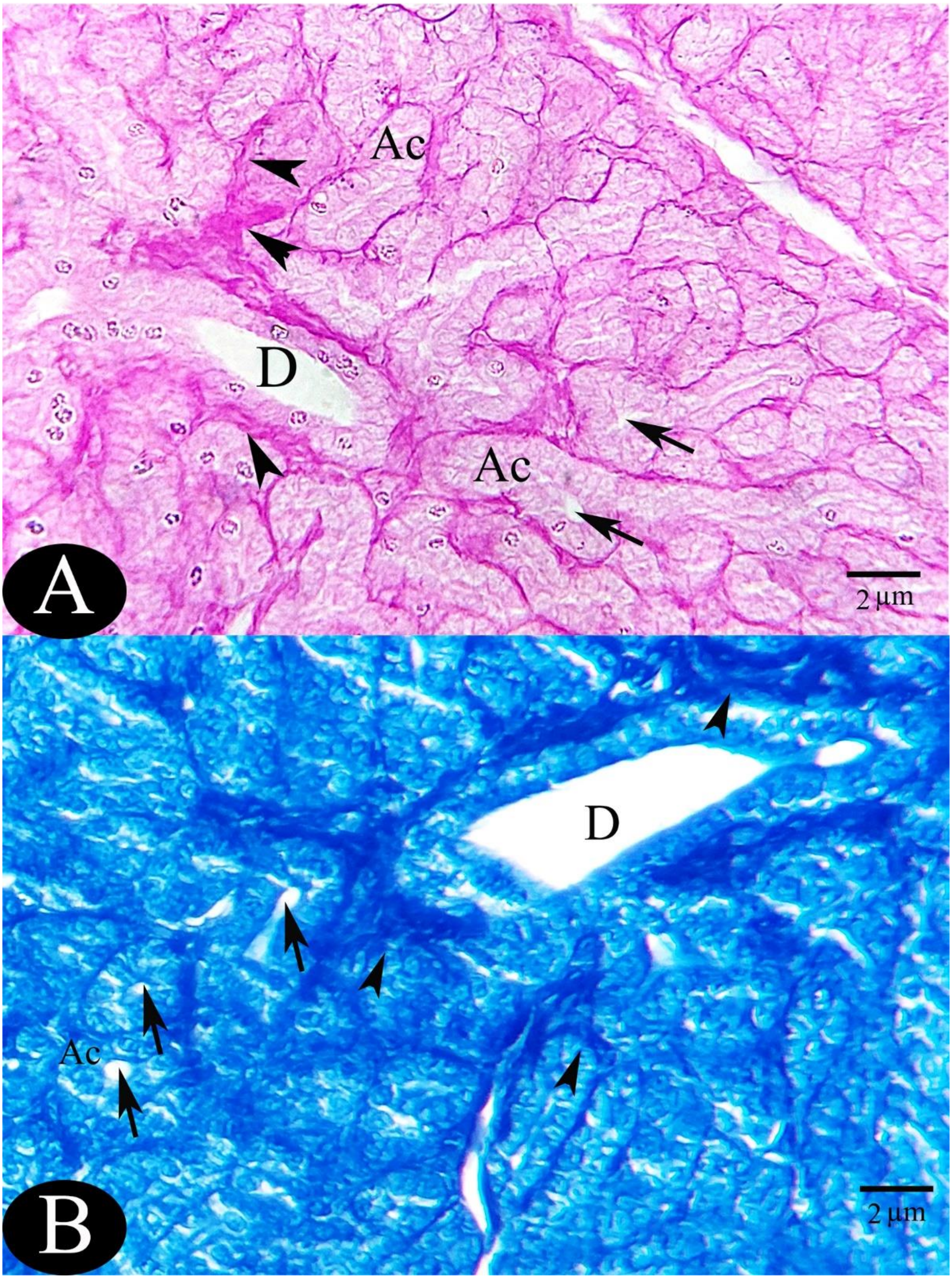

Figure 4: Photomicrograph of a transverse section of the dorsal lacrimal gland of common moorhen, *Gallinula chloropus*, showing: **(A)** the columnar cells of acini (Ac) give positive reaction with periodic acid Schiff's stain (arrow head), the main duct (D), and the secondary duct (arrow) (scale bar = 2 µm), **(B)** the columnar cells of acini (Ac) fill with acid mucin (arrow head), the main duct (D), and the secondary duct (arrow) (Alcian blue, pH = 2.5, scale bar = 2 µm).

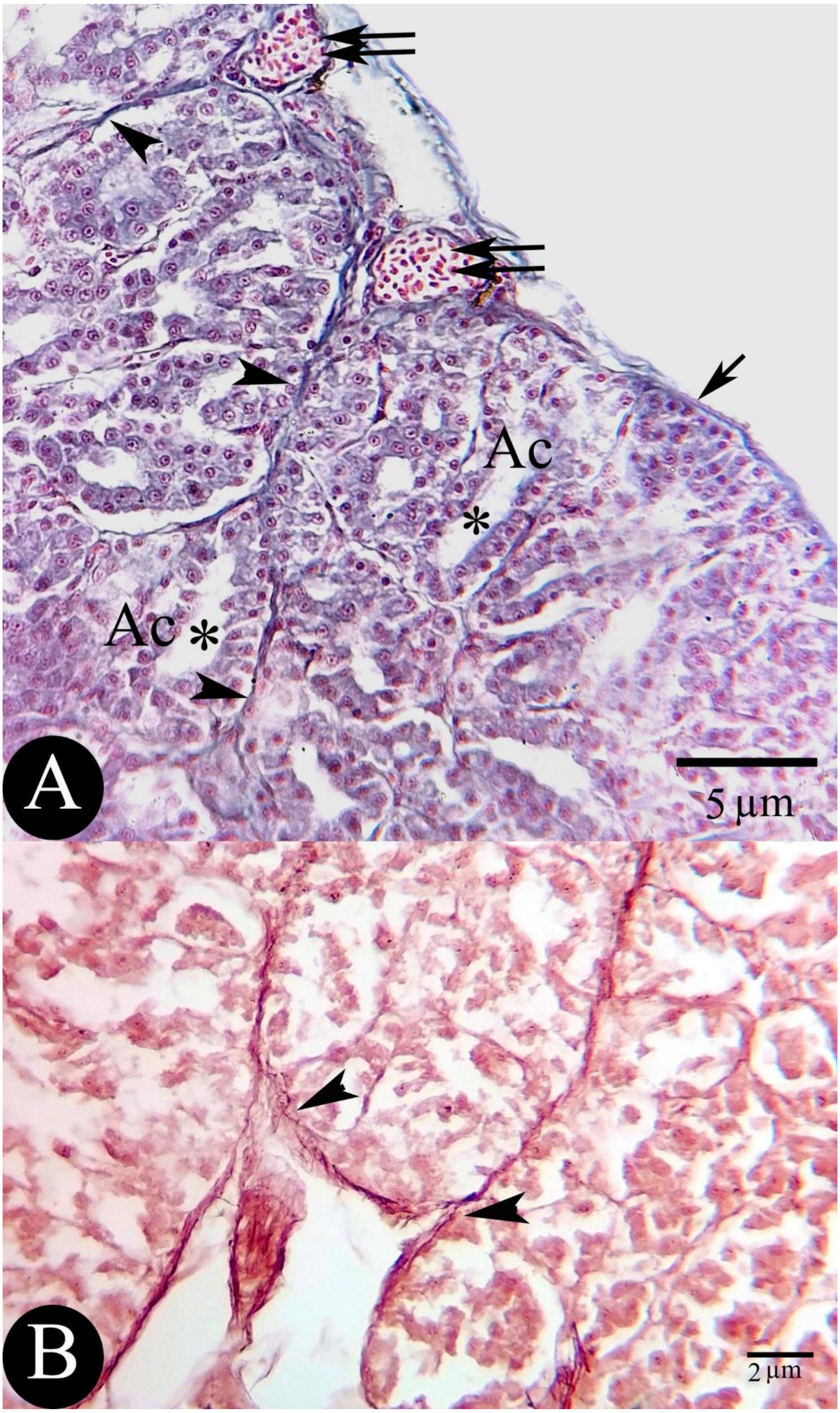

Figure 5: Photomicrograph of a transverse section of the lateral lacrimal gland of common moorhen, *Gallinula chloropus*, showing: **(A)** the lateral lacrimal gland is composed of tubulo-alveoli acini (Ac) and surrounded by a thin capsule of collagen fibers (arrow), the blood vessels (double arrows), and the secondary duct (star) (Masson's trichromic stain, scale bar = 5 µm), **(B)** the capsule rich by elastic fibers (arrow head) (Orcien stain scale bar = 2 µm).

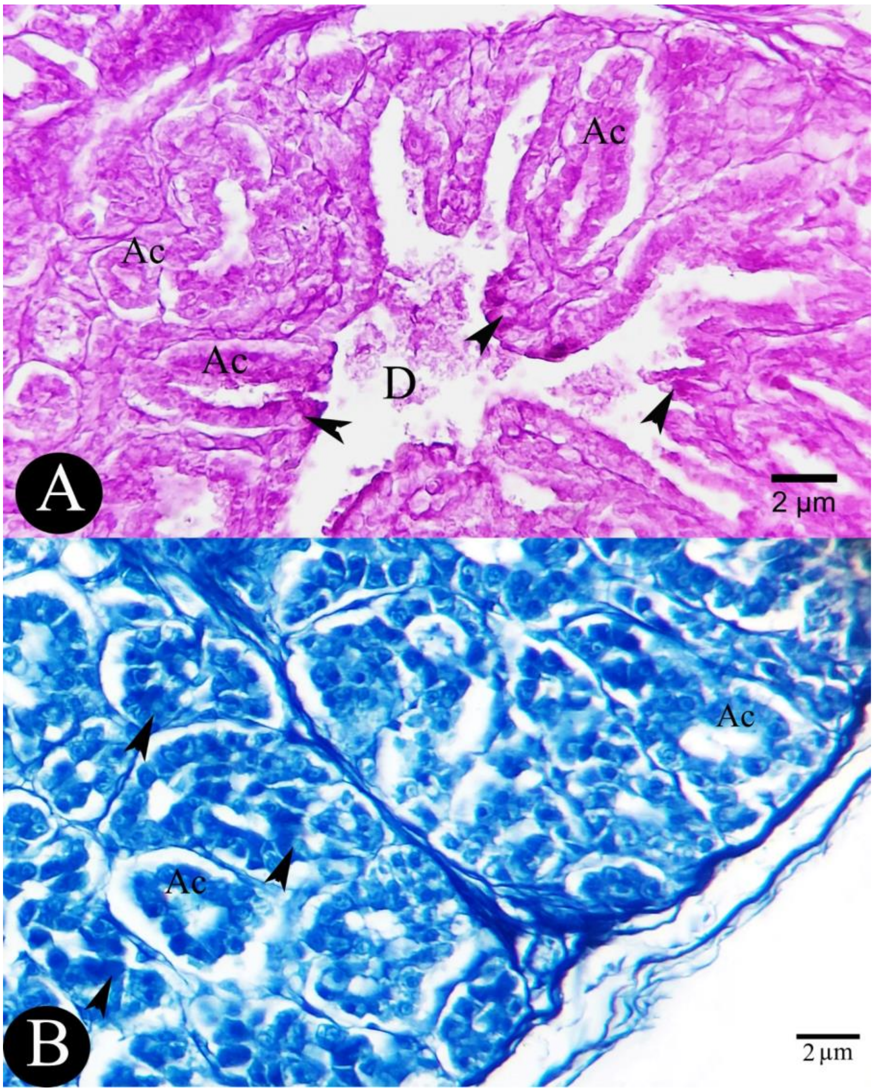

Figure 6: Photomicrograph of a transverse section of the lateral lacrimal gland of common moorhen, *Gallinula chloropus*, showing: **(A)** the cuboidal cells of acini (Ac) give weak positive reaction with periodic acid Schiff's stain (arrow head) and the main duct (D) (scale bar = 2 µm), **(B)** the cuboidal cells of acini (Ac) fill with acid mucin (arrow head) (Alcian blue, pH = 2.5, scale bar = 2 µm).

Description of Harderian gland

The anatomical observation of the Harderian gland of the common moorhen, *Gallinula chloropus*, revealed that the Harderian gland is a voluminous-irregular-lobed gland with pale pink in color and little black patches near its draining duct (Figure 7). This gland occupies an extremely large space of the orbit at the ventromedial surface of the eyeball hidden completely by the depressor palpebral inferioris muscle (Figure 8A). The Harderian gland of the common moorhen consists of three lobes; anterior, posterior, and ventral lobes. The anterior lobe of the Harderian gland appears to be the largest in size, facing the interorbital septum and hiding the distal-third part of the rectus medialis muscle. The ventral lobe of this gland takes the shape of a horseshoe that extends posteriorly, constituting a groove that allows passage of the optic nerve. Furthermore, the ventral lobe and the anterior lobe of the Harderian gland form a shallow groove, and the oblique ventralis muscle runs over this groove. Meanwhile, the posterior lobe of the Harderian gland is sandwiched between the rectus medialis muscle and the anterior part of the quadratus muscle (Figures 8 and 9). The secretion of the Harderian gland is emptied *via* one duct that opens into the anterior conjunctiva fornix just nearly to the base of the nictitating membrane (Figure 9).

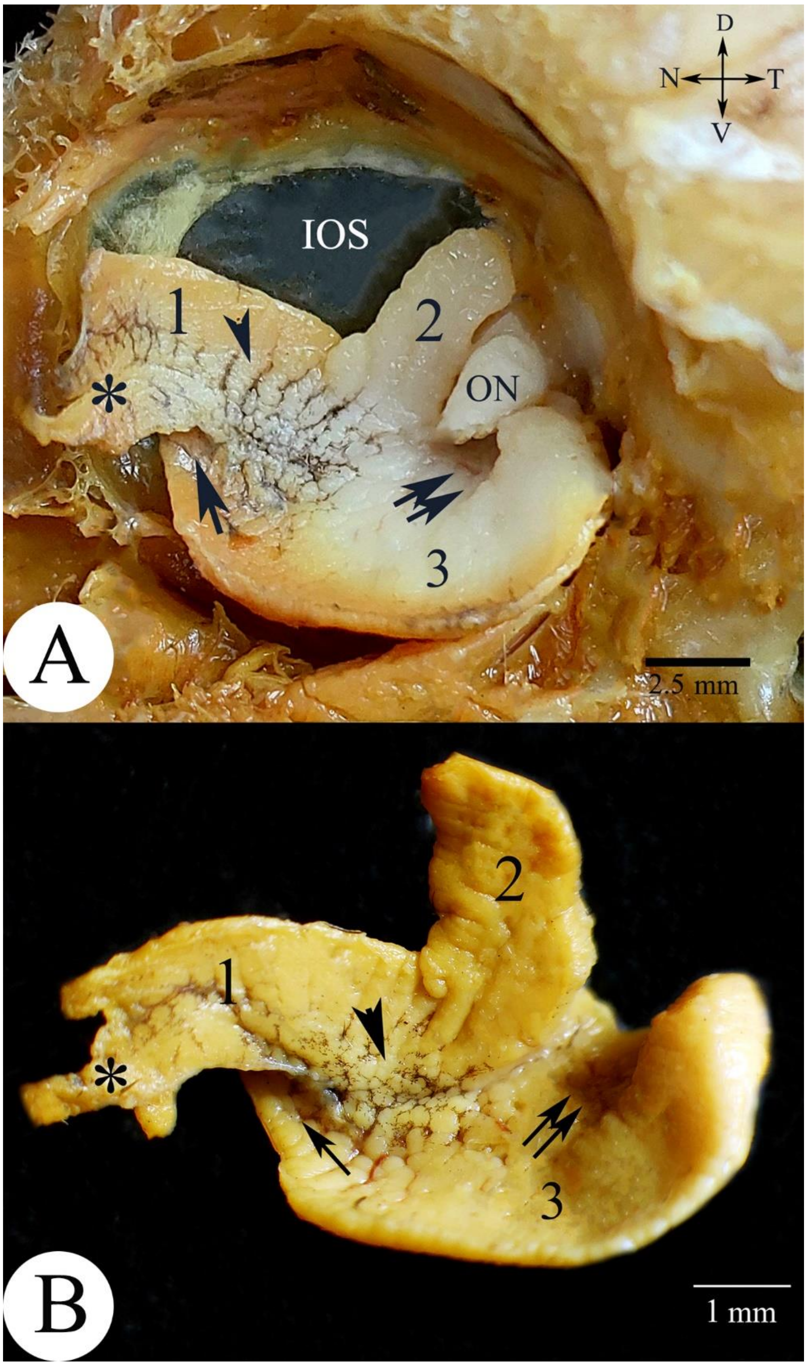

Figure 7: Photomacrograph of Harderian gland of common moorhen, *Gallinula chloropus*, showing: **(A)** the head after removing the eyeball to show the Harderian gland within the orbit facing the interorbital septum (IOS), its lobes: the anterior lobe (1) and posterior lobe (2), and ventral lobe (3) forming a groove (double arrows) on which the optic nerve (ON) passes to reach the eyeball, the anterior lobe (1) and ventral lobe (3) of the gland forming groove (single arrow) and many dark pigmented patches (arrow head), and the harderian gland extends anteriorly to form the main duct (star) (scale bar = 2.5 mm), **(B)** isolated Harderian gland has the three lobes (1,2 and 3) with its main duct (star) and many patches of dark pigmentation (arrow head) (scale bar = 1.0 mm).

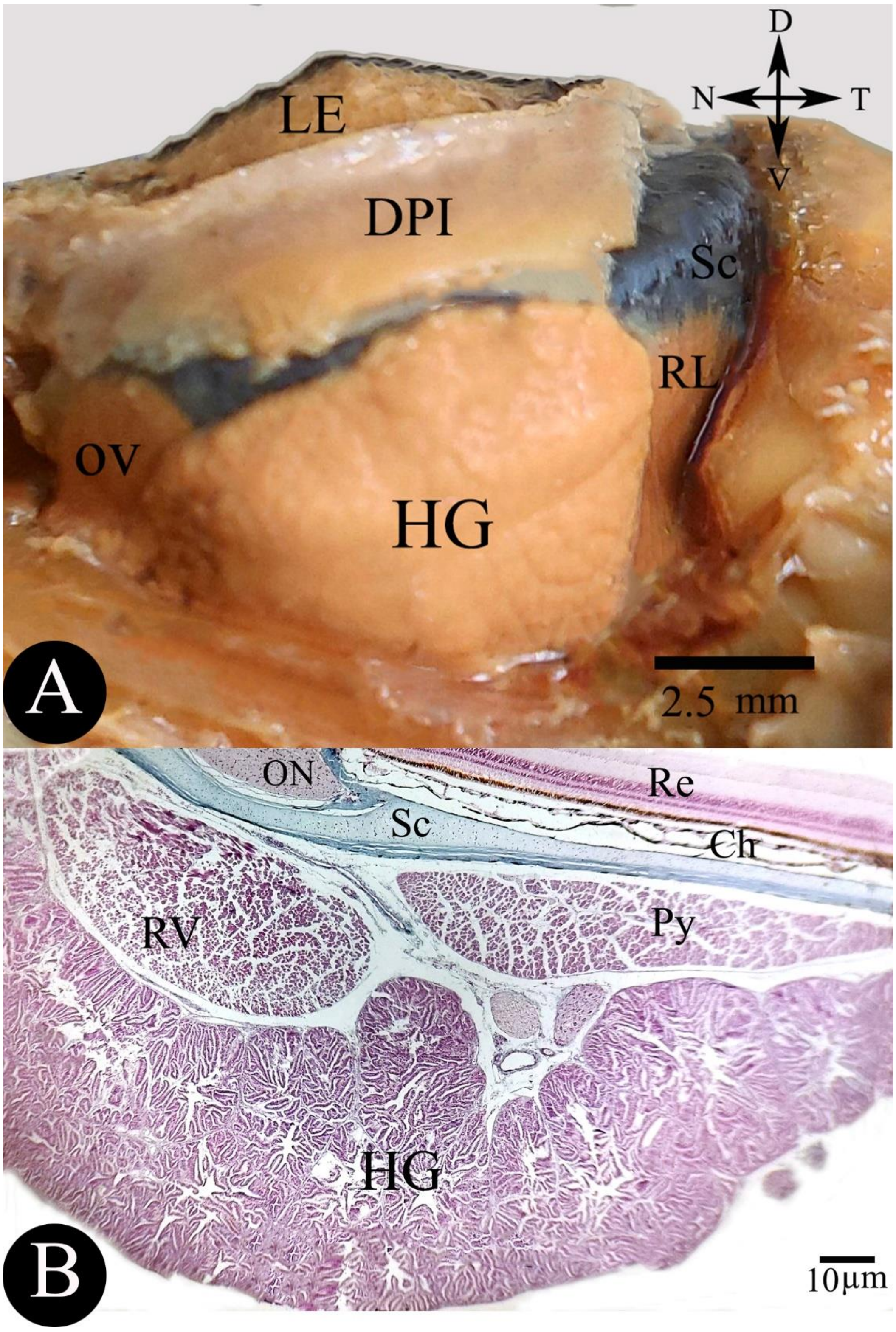

Figure 8: Ventral view in the eye of common moorhen, *Gallinula chloropus*: **(A)** photomacrograph after partially cutting of the depressor palpebral inferioris muscle (DPI), showing the Harderian gland (HG), oblique ventralis muscle (OV), and rectus lateralis muscle (LR) (scale bar = 2.5 mm), **(B)** photomicrograph of a transverse section of the Harderian (HG) showing the rectus ventralis muscle (VR) and the pyramidalis muscle (Py), optic nerve (ON), sclera (Sc), choroid (Ch), and retina (Re) (Masson's trichromic stain, scale bar = 10 μm).

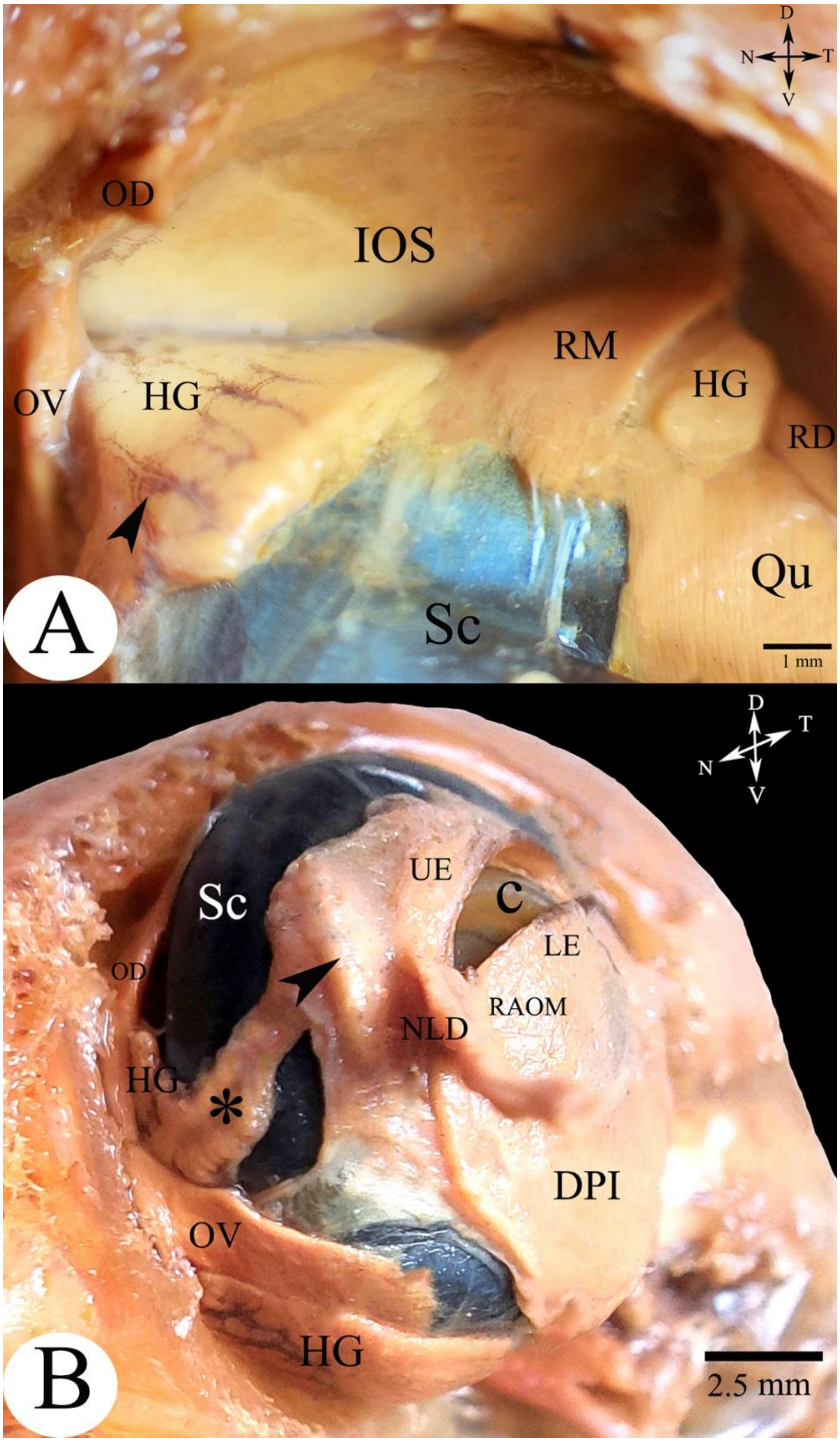

Figure 9: Photomacrograph of the eye of common moorhen, *Gallinula chloropus*, showing: **(A)** a dorsomedial view of the Harderian gland with many dark pigmented patches (arrow head), quadrates muscle (Qu), rectus dorsalis muscle (DR), rectus mediales muscle (MR), oblique dorsalis muscle (OD), optic nerve (ON), interorbital septum (IOS), and the sclera (Sc). (scale bar = 1 mm), **(B)** the draining duct of harderian gland opens into the anterior conjunctival fornix under the nasolacrimal duct (NLD), oblique dorsalis muscle (OD), oblique ventralis muscle (OV), depressor palpebral inferioris muscle (DPI), retractor anguli oculi medialis (RAOM), sclera (Sc) and the cornea (C) (scale bar = 2.5 mm).

Histological investigation reveals that the Harderian gland of the common moorhen, *Gallinula chloropus,* is a compound tubulo-acinar gland enclosed in a thin capsule of connective tissue. This capsule radiates fine septa to divide the parenchyma of the gland into lobules of varied sizes and shapes. Myoepithelial cells can be observed in the interstitial space. Blood vessels and nerve fibers pass through the capsule and its septa. Each lobule of the Harderian gland contains secretory units of acini. Each acini consists of cuboidal cells with large round nuclei (Figure 10). The acinar cells exhibit a positive reaction with the PAS stain and the Alcian blue (pH = 2.5) stain (Figure 11).

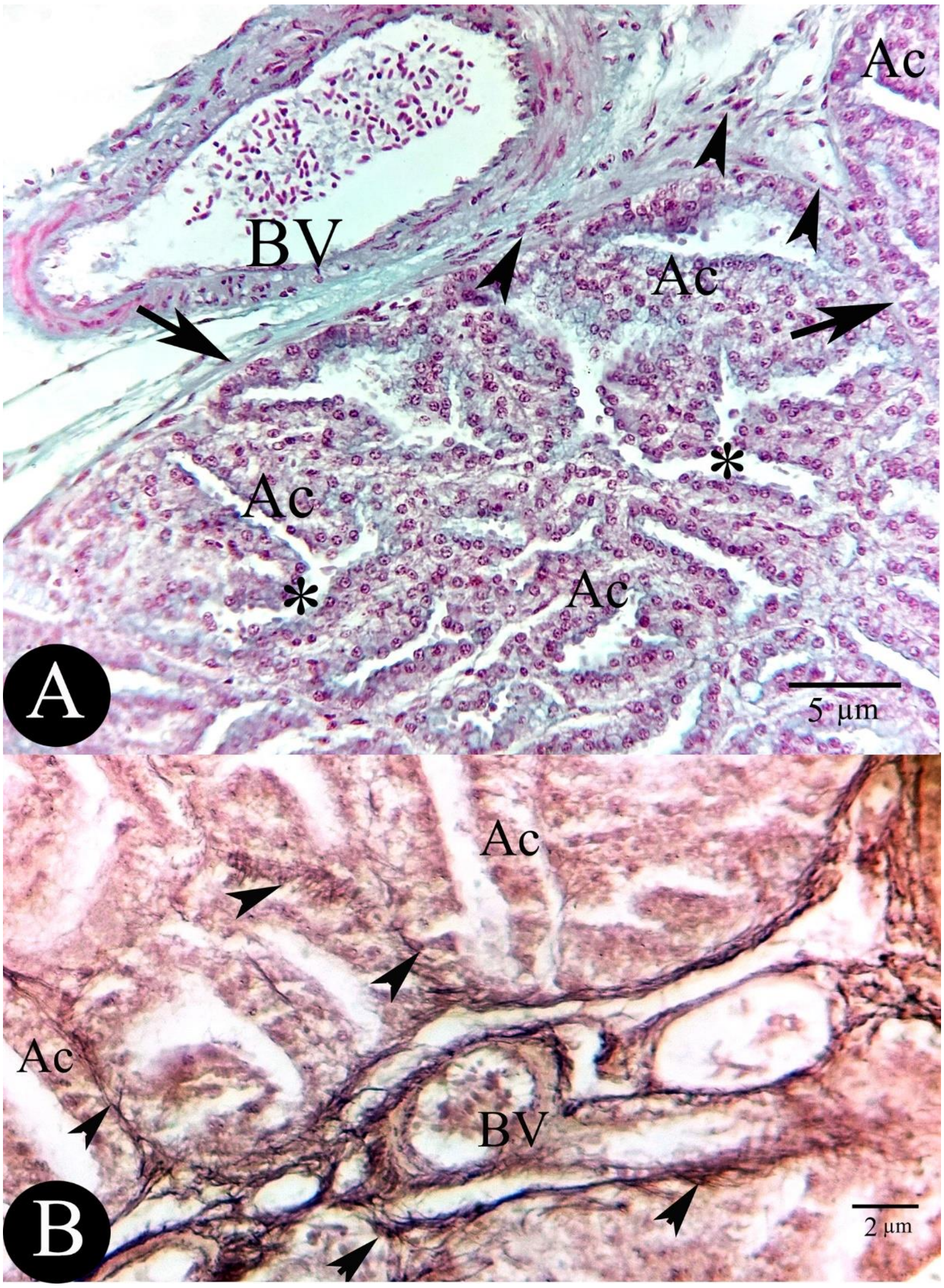

Figure 10: Photomicrograph of a transverse section of the Harederian gland of common moorhen, *Gallinula chloropus*, showing: **(A)** the harderian gland is composed of tubulo-alveoli acini (Ac) lined by cuboidal cells and surrounded by a thin sheet of collagen fibers (arrow), myoepithelial cells (arrow head), secondary duct (star) and blood vessels (BV) (Masson's trichromic stain, scale bar = 5 µm), **(B)** the capsule rich by elastic fibers (arrow head) and blood vessels (BV) (Orcien stain scale bar = 2 µm).

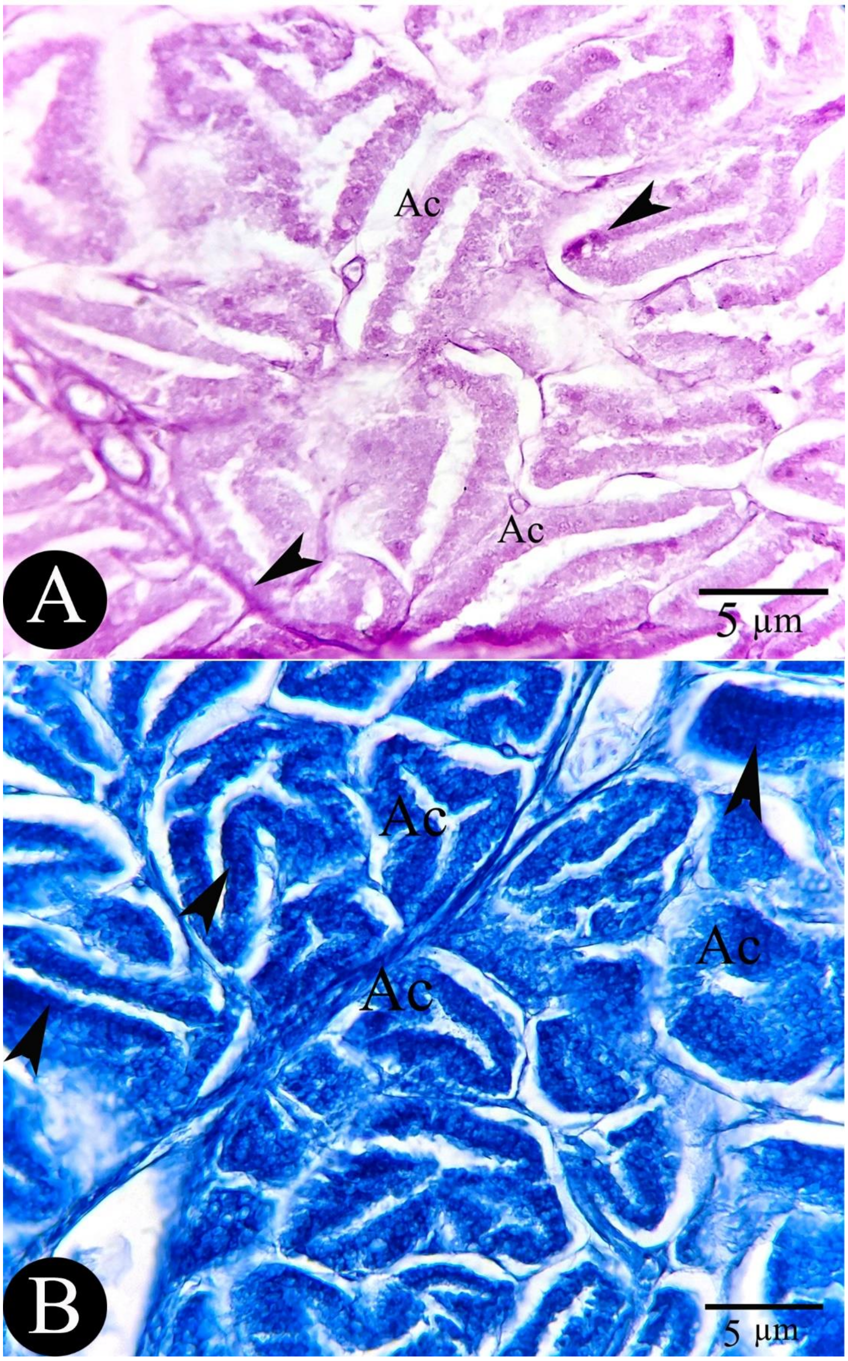

Figure 11: Photomicrograph of a transverse section of the harderian gland of common moorhen, *Gallinula chloropus*, showing: **(A)** the cuboidal cells of acini (Ac) give a positive reaction with periodic acid Schiff's stain (arrow head) (scale bar = 5 µm), (B) the cuboidal cells of acini (Ac) fill with acid mucin (arrow head) (Alcian blue stain, pH = 2.5, scale bar = 5 µm).

DISCUSSION

The present work deals with the orbital glands in common moorhen to coordinate their location and structure, as well as, their function, with the habitat of that bird. The present anatomical investigation exhibited great differences in the features of the orbital glands of common moorhen compared to different avian species. The common moorhen possesses dorsal and lateral lacrimal glands that differ in size, location, and shape. Several studies of avian ophthalmology concluded that only one lacrimal gland has appeared in the avian eye dorsal or lateral but not two, for example, in the cattle egret[4], only a small lacrimal gland with a drop-like shape appeared at the posterior pole of the eye; while in other avian species such as the little owl and hoopoe[2,4], only the dorsal lacrimal gland has been observed.

Reese *et al*.[36] mentioned that the nasal gland is a modified lacrimal gland located dorsally within the orbit. In certain seabirds, it functions as osmoregulation for salt excretion. Therefore, it is widely mentioned that the dorsal lacrimal gland, or nasal gland, in some avian species, e.g., the common moorhen that lives near the sea, was modified into a salt gland for osmoregulation function. Thus, the salt gland in seabirds is homologous to the dorsal lacrimal gland in the present studied bird species. However, the lateral lacrimal gland of the common moorhen has been found in some birds, e.g., English sparrow[37], turkey, and chicken[8,38].

Furthermore, the common moorhen possesses a voluminous Harderian gland with an irregular multi-lobed shape, like that in the English sparrow[37]. It may also be roughly triangular, as in the domestic fowl[39]. On the other hand, many birds have flat Harderian glands that resemble teardrops, as found in the osprey[40], pigeon[19], and the little owl[2]. However, the shape of this gland in the avian species exhibits great variety, its position is very limited. In all avian species, the Harderian glands are situated venteromedially to the eyeball, but some may be more anterior than others. In many other birds, the Harderian gland is situated anteroventromedially between the oblique ventralis and pyramidalis muscles[2,7]. The present anatomical observation revealed that the Harderian gland of the common moorhen is situated ventromedially to the eyeball and covers almost one-third of the posterior surface of the eye, where it passes under the proximal insertions of the oblique dorsalis and oblique ventralis muscles. The location of the Harderian gland in the common moorhen between two eye muscles, makes a pressure on the body of the gland, that facilitates its secretion into the anterior surface of the cornea and the nictitating membrane. Additionally, the body of this gland acts as a hydrostatic cushion for these eye muscles.

The current histological investigations reveal that both the Harderian and lacrimal glands of the common moorhen are compound tubulo-alveolar glands. Based on this finding, the Harderian gland in the common moorhen can be classified as type I in accordance with Burns' classification[12] of the avian Harderian gland, who classified it into three distinct types based on the acinar type and lobular epithelium structure. Similar results were described in the little owl[2], ostrich[5], quail[14], capercailis[7], and in cattle egret and hoopoe[4].

However, Aitken and Survashe[41] suggested that segregation of gland types is obvious when avian species are assorted depending on their environments. A type II gland is obviously not present in any terrestrial species, but aquatic birds may have either a type II or type III gland, indicating that type II lobules may be correlated to the aquatic habitat. Furthermore, the histochemical analysis performed on the orbital glands of the common moorhen confirmed the presence of acid mucins. The nature of both orbital glands' secretions in the common moorhen indicates their protective function in eye disorders, as does the typical function of lubrication of the anterior surface of the eyeball and nictitating membrane[15]. Shawki *et al.*[2] mentioned that the structure of eyelids, the position of orbital glands, and the nature of their secretion play a significant role in determining the optical quality of the cornea, thereby directly affecting the quality of the retinal image. According to Al-Nefeiy *et al.*[4], there is a correlation between the anatomical features of the lacrimal gland in hoopoe and cattle egret the requirements of their environments, and suggested that the mucins of the lacrimal gland play a significant role as anti-inflammatory and antibacterial agents, avoiding microbial growth in the nasal cavity and on the cornea surface.

In conclusion, the common moorhen is an aquatic bird that lives around well-vegetated marshes, ponds, canals, and other wetlands, and possesses two lacrimal glands (one of which opens into the nasal cavity and the lateral lacrimal opens into the posterior conjunctival fornix) and a voluminous Harderian gland. In addition, the present study suggested that the acidic secretion of both orbital glands, along with the increasing size of the Harderian gland and number of lacrimal glands, increased the effective protection of the eye.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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دراسة تشريحية ومجهرية للغدد العينية لدجاجة الماء "*GALLINULA CHLOROPUS*"

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تم إجراء العديد من الدراسات المختلفة حول الغدد العينية للطيور. ومع ذلك، فالمزيد من الدراسات المقارنة التي تشمل العديد من الأنواع تحتاج إلى اهتمام أكثر. تهدف الدراسة الحالية إلى دراسة العلاقة بين الخصائص المورفولوجية والنسيجية والكيميائية النسيجية للغدد العينية لدجاجة الماء (*Gallinula chloropus*) ووظيفتها الوقائية للعين. توجد غدتان دمعيتان في دجاجة الماء، تختلفان في الحجم والشكل والموقع وموضع فتح كل منهما. تظهر الغدة الدمعية الظهرية كغدة اسطوانية ثنائية الفصوص ذات شكل انضغاطي ظهري بطني تقع داخل الحفرة في الحافة الحجاجية الظهرية وتتحد من الأمام أسفل العظم الدمعي لتشكل قناة التصريف الخاصة بها. والغدة الدمعية الجانبية صغيرة تقع في القطب الخلفي لمقلة العين. وتبدو غدة هاردر ضخمة الحجم وذات شكل غير منتظم ومتعدد الفصوص. ومن الناحية النسيجية، تتكون الغدد العينية من الطرز الأنبوبي السنخي المركب، حيث تبطن كل أسيني بخلايا مكعبة ذات أنوية مستديرة كبيرة ومغطاة بمحفظة مصبوغة. وتكشف هذه الغدد عن تفاعلات إيجابية مختلفة مع كاشف "Schiff" وصبغة "Alcian blue" (الرقم الهيدروجيني = 2.5). وخلصت الدراسة الحالية إلى أن دجاج الماء يمتلك طرزًا مختلفة من الغدد العينية: غدتان دمعيتان وغدة هاردر الضخمة. ورجحت الدراسة الحالية أن الغدد العينية تغير من سماتها عند هذا الطائر الذي يلتقط طعامه من تحت الماء لضبط الانكسار البصري المائي؛ علاوة على ذلك، فإن هذه الغدة بحكم إفرازاتها الحمضية قد تسمح بحماية العين من أي تلوث مائي.