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ANATOMICAL, HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF ORBITAL GLANDS OF LITTLE OWL *ATHENE NOCTUA*

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The present study aimed to illustrate the anatomical, histological and histochemical structure of the two orbital glands of the little owl, *Athene noctua* with revealing their role in the vision. In little owl, the size of lacrimal gland appears smaller than that of the Harderian gland. The Harderian gland of the little owl is flat and looks like teardrop in shape with pink color and located between the ventralis oblique muscle and pyramidalis muscle. While, the lacrimal gland is cylindrical, compressed in the dorsal-ventral direction and is located on the dorsal edge of the orbit within a narrow elongated fossa. Both glands are compound tubuloalveolar type. The acini of Harderian gland are lined by cuboidal glandular cells whereas the lacrimal gland acini are lined by columnar cells. The Harderian gland in the little owl is classified as a type I. The Harderian gland and lacrimal glands of the little owl contain neutral and acid mucosubstances.

INTRODUCTION

The avian eye possesses two types of glands; Harderian and lacrimal glands. These orbital glands play an important role in lubrication of the cornea surface and nictitating membrane (Payne 1994), while they also serves as a source of growth hormones (Seyama

and Uchijama 2007; Baccari et al. 1993) and play a role in the immune response (Ohshima and Hiramatsu 2002; Oliveria et al. 2006). Many previous studies had been done on the avian Harderian gland (Maxwell et al 1986; Altunay and Kozlu 2004; Kozlu and Altunay 2011; Kleakowska-Nawrot et al. 2015; Reshag et al. 2016) compared to the lacrimal glands (Kleakowska-Nawrot et al. 2015; Kleakowska-Nawrot et al. 2016; Reem and Khattab 2018). The rare of comprehensive information on the anatomy of the lacrimal gland in birds is due to its minor importance in eye physiology when compared with the Harderian gland. The components of the lacrimal secretory system are part of the tear film (Kleakowska-Nawrot et al. 2016). In all terrestrial vertebrates, the lacrimal gland is responsible for the secretion of tear fluid which together with conjunctiva-associated lymphoid tissue, helps to maintain corneal health (Mohammadpour 2009) and also it has an important role in eye physiology and pathology (Kawahima et al. 2012).

The structure of these orbital glands of the little owl has not been studied previously. Thus, the present study aims to demonstrate the anatomical feature, as well as, the histological and histochemical structures of the two orbital glands in little owl, *Athene noctua.*

MATERIALS and Methods

The samples of Harderian and lacrimal glands were obtained from five adult specimens of little owl. The specimens were dissected according to the guidelines of the research ethics committees, Assiut University (www.enrec.org). The left Harderian and lacrimal glands were dissected and isolated then fixed in 10% neutral formalin, routinely processed and embedding in paraffin wax. Blocks were cut at 7µm thick, and stained with Masson's trichrome for detection of the collagenous fibers and Orcein stain for elastic fibers ((Drury and Wallington 1980). For histochemical study, the sections were stained with PAS reaction for general polysaccharides and Alcian blue pH 2.5, for mucopolysaccharides respectively (Bancroft and Stevens 1996).

RESULT

The eye of the little owl contains two types of orbital glands; lacrimal and Harderian gland

Morphological observations of the lacrimal gland

The lacrimal gland of the little owl is pink in color, cylindrical in shape and slightly dorso-ventrally compressed (Fig.2). The lacrimal gland situates on the dorsal rim of the orbit and enveloped

by the periorbital sheet (Fig. 1). This gland extends anteriorly under the lacrimal process and penetrates the lateral process of the ectoethmoid bone to drain their secretion into the nasal cavity via unpaired duct (lacrimal canaliculi) (Fig.3)

Histological and Histochemical observations of the lacrimal gland

The lacrimal gland is a compound branched gland of tubuloalveolar type. The acini of lacrimal gland are formed of columnar cells with oval nuclei lying in their basal portions (Fig. 4). The lacrimal gland is encased inside a connective tissue sheath rich in elastic fibers than collagenous fibers (Fig. 5). This sheath extends to divide the gland into lobes and small lobules (Figs. 4& 5). The secretory portions of this gland are filled with granular basophilic and vacuolated cytoplasm which gives deep bluish color with alcian- blue at pH 2.5 that indicates a strong reaction for acid mucin while gives weak reaction with PAS reaction (Figs. 6&7).

Morphological observation of the Harderian gland

The Harderian gland of the little owl is flat and elongated flask-shaped gland with light pink color (Fig. 8). This gland locates on the antero-ventro-medial surface of sclera cartilage, ventral to the ventral oblique muscle and dorsal to the pyramidalis muscle (Fig.9).

The duct of Harderian gland extends medially towards the medial canthus and opens at the medial edge of the nictitating membrane.

Histological and Histochemical observations of the Harderian gland

The Harderian gland of the little owl seems as a compound and branched gland of tubuloalveolar type. The acini of this gland consist of cuboidal cells with large rounded nuclei (Fig. 10) The Harderian gland is surrounded by thin collagenous connective tissue capsule which penetrates this gland to divide it into elongated lobes of varying sizes (Fig.10). The glandular cells give deep bluish color with alcian-blue at pH 2.5 that indicates a strong reaction for acid mucin while giving weak reaction with PAS reaction (Figs. 11& 12).

DISCUSSION

The present study is focused on the anatomical, histological and histochemical structure of the lacrimal and Harderian glands of the little owl.

The morphological observations of the lacrimal and Harderian glands of the little owl revealed that the lacrimal gland is smaller than the Harderian gland. The differences in the size of the orbital glands were observed in many avian species e.g. in Japanese quails (Dimitrov and Genchev 2011), in Bilgorajska goose (Kleakowska-

Nawrot et al. 2016) and in Ostrich (Reem and Khattab 2018). Reem and Khattab (2018) observed that the Harderian gland was much larger in size than the lacrimal gland in ostrich

The Harderian gland of the little owl is flat and looks like teardrop in shape with pink color. These morphological features of this gland in little owl were similar to those reported by Kozlu et al. (2010) in osprey and Reshag et al. (2016) in pigeon. While in fowl and sparrow, this gland appeared irregular in shape (Payne 1994) and hemispherical shape in ducks and pelican (Burns 1992)

The Harderian gland of the little owl locates antero-ventromedially between the oblique ventralis and pyramidalis muscle. This location was similar to that of other avian species (Mobini 2014; Kleakowska-Nawrot et al. 2016; and Reshag et al. 2016). This result has disagreed with Reem and Khattab (2018) who mentioned that the Harderian gland in ostrich was situated in orbit, lateral to the caudal portion of the interorbital septum and medial to the caudal third of the eyeball.

The present study reveals that the lacrimal gland of the little owl is positioned on the dorsal rim of the orbit within narrow-elongated fossa. This location of the lacrimal gland in little owl had not been previously observed in other birds.

Several previous studies on the location of the Lacrimal glands have been carried out in many avian species (Kleakowska-Nawrot et al. 2015; Kleakowska-Nawrot et al. 2016; and Reem and Khattab 2018) and they concluded that the lacrimal gland is positioned in the periorbital space sometimes locate in the dorsotemporal part of the orbits as in owls (Harris et al. 2008) or appear in caudal to lateral canthus of the eye as in ostrich (Reem and Khattab 2018).

But in case of the little owl, the present authors observed that the lacrimal gland stabilizes over bone and its dorsal surface is covered completely by the periorbital sheet. This periorbital sheet is connected tightly with the capsule of this gland. Therefore, there is a relationship between the properties of this gland in the little owl and its drainage function that happens through the cooperative actions between the outer fibrous sheet and its capsule.

In little owl, the lacrimal gland is cylindrical in shape with slightly dorsoventrally compression. This result is similar to the description of the lacrimal gland in Capercaillies which was mentioned by Kleakowska-Nawrot et al. (2016); this gland in Capercaillies appeared uniform and undivided and flattened in shape.

Furthermore, the histological investigation of the Harderian and lacrimal glands of the little owl revealed that both glands are compound tubuloalveolar glands. From this result, the Harderian gland in the little owl can be classified as a type I according to the classification of Burns (1992) who classified the avian Harderian gland into three different types according to the acinar structure. Similar findings were described in quail (Kozlu and Altunay 2011), in Capercailis (Kleakowska-Nawrot et al. 2016) and in ostrich (Reem and Khattab 2018).

The present study reveals that the Harderian and lacrimal glands of the little owl are composed of alveoli/acini that consist of cuboidal in Harderian gland whereas in lacrimal gland, the acini are composed of columnar cells. Frahmand and Mohammadpour (2015) and Reem and Khattab (2018) mentioned the structure of Harderian gland in Ostrich and Canadian ostrich respectively. They reported that the acini of Harderian gland are composed of tall columnar epithelial cells with basal oval nuclei. Meanwhile, Kleakowska-Nawrot et al. (2016) recorded that the Harderian and lacrimal glands of Capercailis were composed of pyramidal cells with slightly granular basophilic cytoplasm and reported that these results agree

with those mentioned by Kozlu et al. (2010) in osprey and Kozlu and Altunay (2011) in quail.

Analysis of the histochemical results demonstrates that the secretion products of Harderian gland and lacrimal glands of the little owl contain neutral and acid muco-substances. These results indicate that the secretion of Harderian gland and lacrimal glands of the little owl contains carboxylated acidic mucopolysaccarides. The nature of secretion of both orbital glands in little owl indicates their protective role in the ocular disorder, in addition to the usual function of these glands which is lubrication of the surface of the eyeball and nictitating membrane (Mobini 2012)

Interestingly, the histochemical features of the Harderian gland and lacrimal glands resemble those of the fowl (Wright et al. 1971), ducks (Dimitrov and Nikiforov 2005), in capercaillie (Kleakowska-Nawrot et al. 2016) and turkeys (Khayoon et al. 2019) in which the carboxylated acid mucopolysaccharides appear in both glands. They were found the nature of secretion of these glands confirms their role in the production of the tear film to lubricate the ocular surface and nictitating membrane as well as their immune protective role in the ocular disorder.

CONCLUSION

The morphological and histological study of the Harderian and lacrimal glands in little owl exhibit great similarities and little differences with other avian species. The lacrimal gland of little owl is smaller than the Harderian gland. Moreover, the Harderian gland is flat and looks like teardrop in shape with pink color and is located antero-ventromedially between the oblique ventralis and pyramidalis muscle. Meanwhile, the lacrimal gland is cylindrical in shape which is slightly dorsoventrally compressed and locates on the dorsal rim of the orbit within narrow-elongated fossa. Both orbital glands of the little owl are compound tubuloalveolar in structure. The alveoli/ acini are composed of cuboidal cells in Harderian gland whereas in the lacrimal gland, the acini are lined by columnar cells. The Harderian gland in the little owl is classified as a type I. Both two orbital glands of the little owl contain neutral and acid muco-substances. The nature of secretion of these glands confirms their role in the production of the tear film to lubricate the ocular surface and nictitating membrane as well as their protective role in the ocular disorders.

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Figure Legends

Fig. 1. A photo of the eye of little owl, *Athene noctua*, showing the lacrimal gland (arrow head) is completely covered by periorbital sheet (Psh). In addition the lacrimal process (Lp).

Fig.2. A photo of the eye of little owl, *Athene noctua*, showing the shape of the lacrimal gland (arrow), and its location on dorsal rim of the orbit.

Fig.3. Lateral view of the eye of the little owl, *Athene noctua*, showing the position of the lacrimal gland and its duct (Lc) which opens in nasal cavity (N), and some accessory eye muscles; Levator palpebral superioris muscle (Lps), Levator anguli oculi muscle (lao), Retractor anguli oculi Lateralis muscle (Raol). In addition, the dermatotemporalis muscle (Dt).

Fig.4. Photomicrograph of a transverse section of the lacrimal gland of little owl, *Athene noctua*, showing the branched tubulo-alveoli, lining by columnar cells (arrow head) and surrounded by myoepithelial cell (arrow). (Masson's trichromic stain, 400 x).

Fig.5.Photomicrograph of a transverse section of the lacrimal gland of little owl ,*Athene noctua*, showing the connective tissue sheath rich with elastic fibers (arrow). (Orcien stain, 400 x).

Fig.6.Photomicrograph of a transverse section of the lacrimal gland of little owl, *Athene noctua*, showing the glandular cells of acini filled with acid mucin. (Alcian blue pH 2.5, 400 x).

Fig.7.Photomicrograph of a transverse section of the lacrimal gland of little owl, *Athene noctua*, showing the collumnar cells give weak reaction with PAS reaction. (PAS reaction, 400 x).

Fig.8. A photo of the Harderian gland of little owl, *Athene noctua*, showing the flattened and elongated flask shaped with light pink color.

Fig.9.Ventral view of the eye of the little owl, *Athene noctua*, after cutting the ventral oblique muscle (Vo) showing the location of the Harderian gland (HG) in addition, Depressor Palpebral Inferioris muscle (Dpi), ventralis rectus muscle (Vr), lateralis rectus muscle (Lr) and tendon of pyramidalis (Pyt).

Fig.10.Photomicrograph of a transverse section of the Harderian gland of little owl, *Athene noctua*, showing that the Hardiran gland is

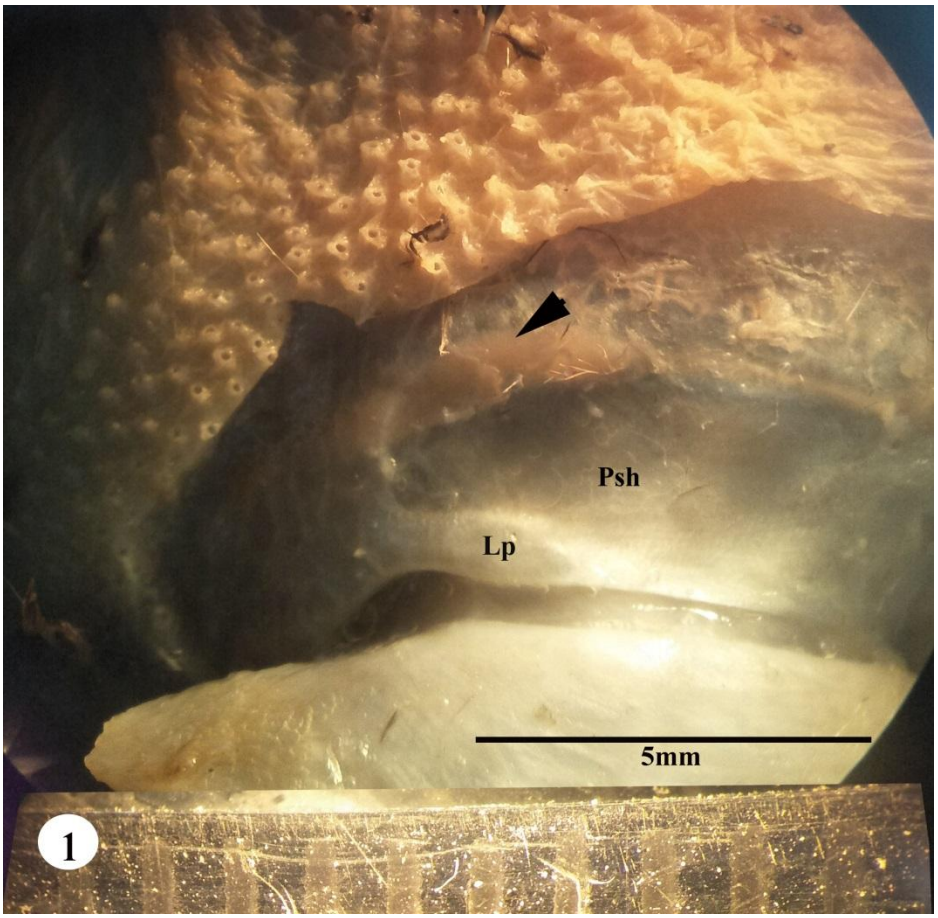
composed of compound and branched tubulo-alveoli acini (arrow) and surrounded by thin collagenous connective tissue capsule which penetrates the gland to divide it into lobes of varying sizes (arrowhead), the acini are lined by cuboidal cells. (Masson's trichromic stain, 400 x).

Fig.11.Photomicrograph of a transverse section of the Harderian gland of little owl, *Athene noctua*, showing the glandular cells with deep bluish color. (Alcian blue pH 2.5, 400 x).

Fig.12.Photomicrograph of a transverse section of the Harderian gland of little owl, *Athene noctua*, showing weak PAS reaction in the glandular cells. (PAS reaction, 400 x).

الملخص العربي

هدفت الدراسة الحالية إلى توضيح التركيب التشريحي والهستولوجي والهستوكيميائي للغدد العينية للبومة الصغيرة معا للكشف عن دورهما في الرؤية. لوحظ ان حجم الغدة الدمعية أصغرمن حجم غدة هارديريان في البومة الصغيرة . تبدو غدة هارديريان مسطحةعلي شكل دمعة باللون الوردي وتقع في الناحية البطنية الأمامية بين العضلة البطنية المائلة والعضلة الهرمية. بينما تكون الغدة الدمعية أسطوانية الشكل منضغطة في الاتجاه الظهري- البطني و مستقرة على الحافة الظهرية للمدارداخل حفرة ضيقة. وجد ايضا ان الغدتان العينيتان للبومة الصغيرة عبارة عن غدد حويصلية – انبوبية مركبة مبطنة بخلايا مكعبة في غدة هارديريان وبخلايا عمادية في الغدة الدمعية . تحتوي غدة هارديريان والغددالدمعية للبومة الصغيرة على مواد مخاطية متعادله و حمضية. وتصنف غدة هارديريان في البومة الصغيرة من النوع (I).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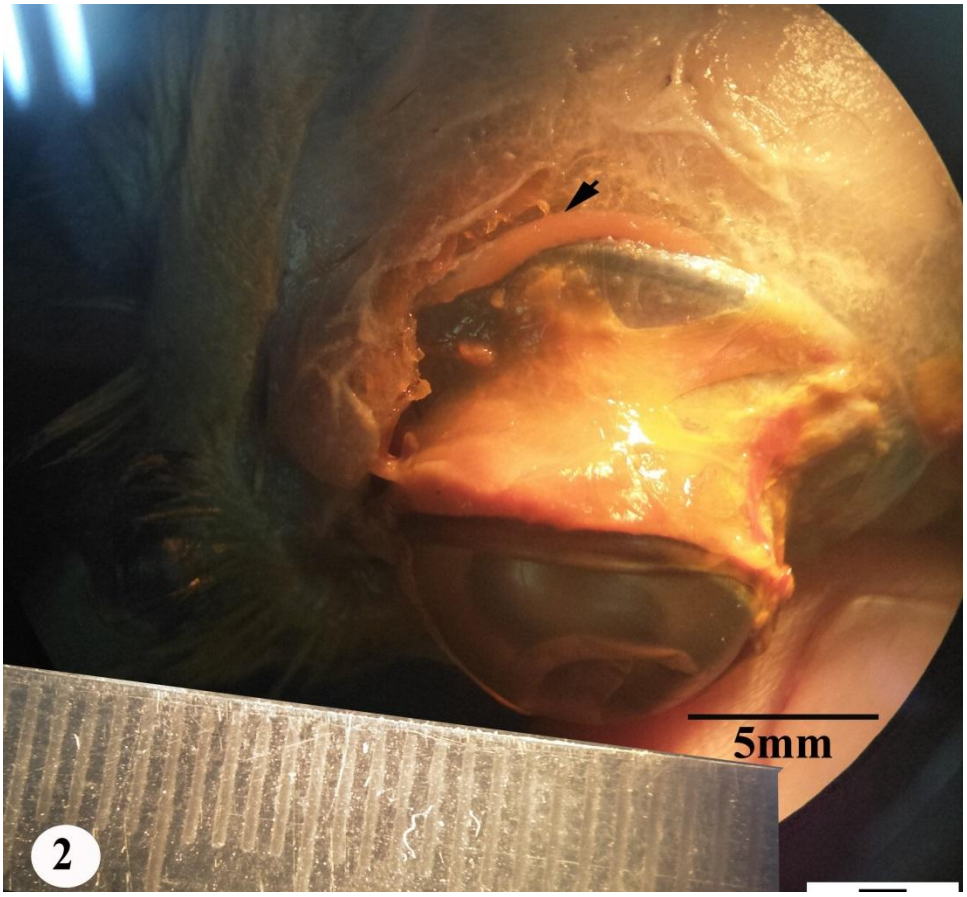
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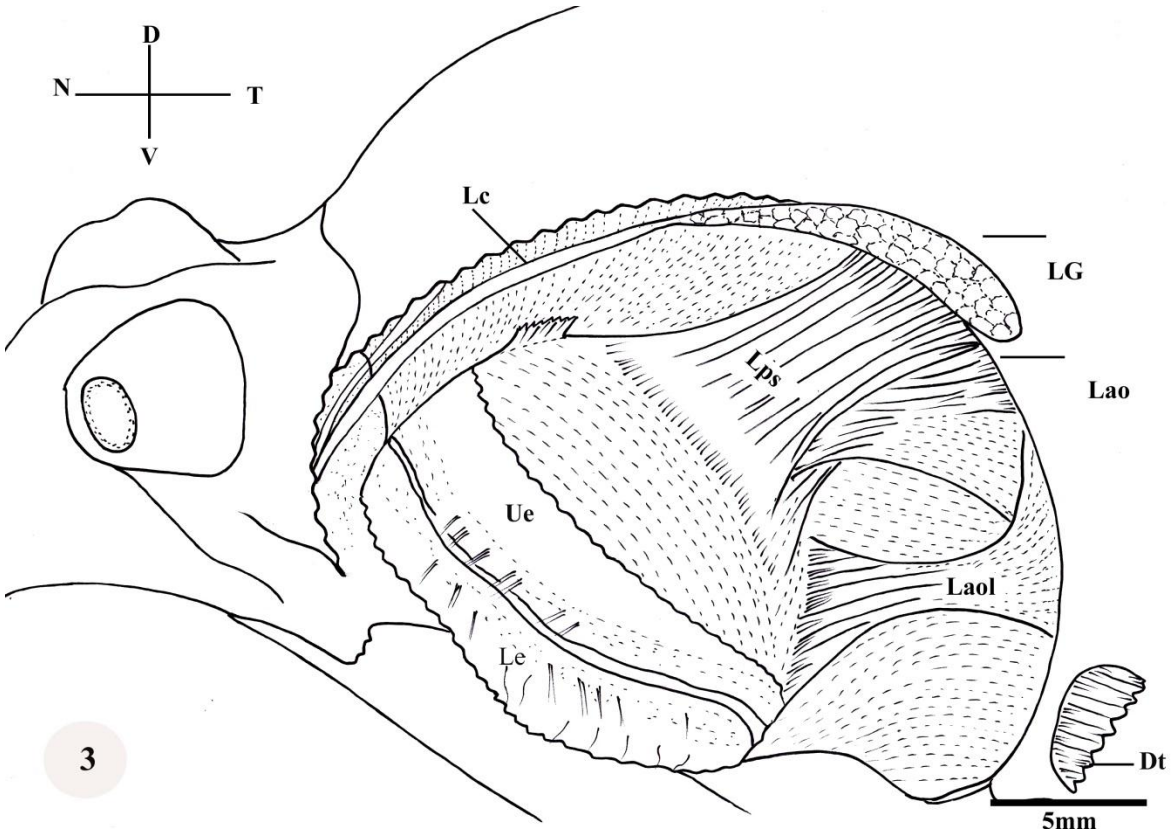
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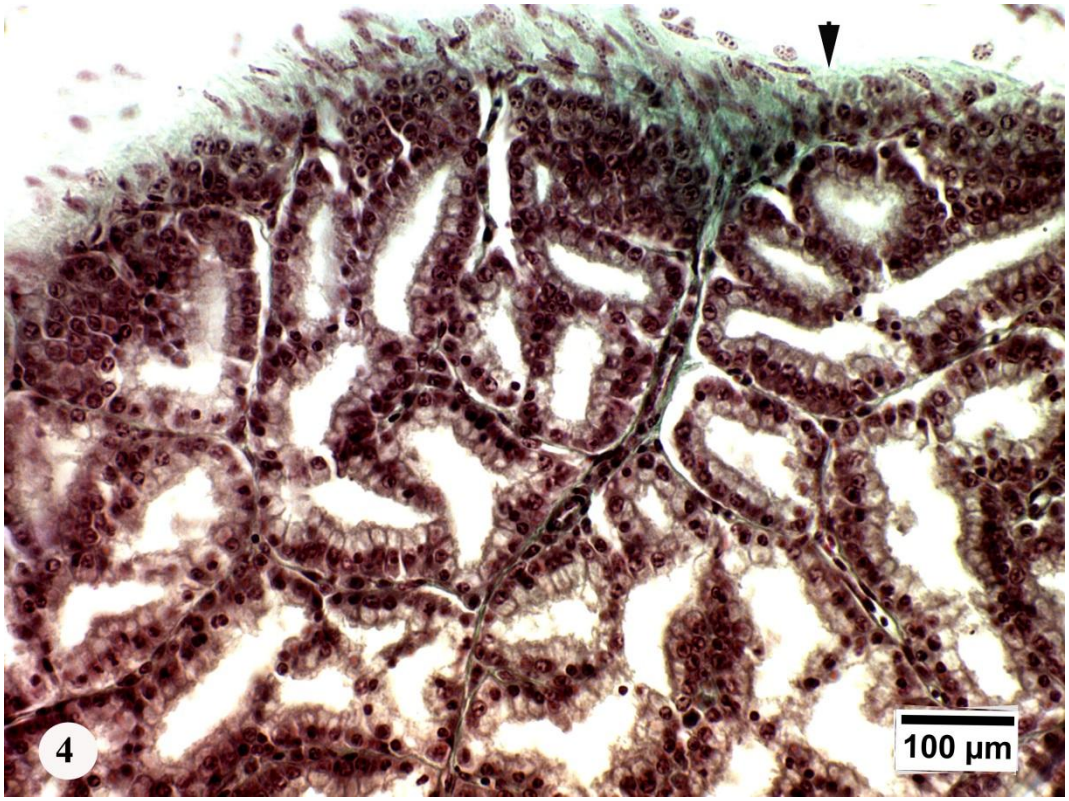
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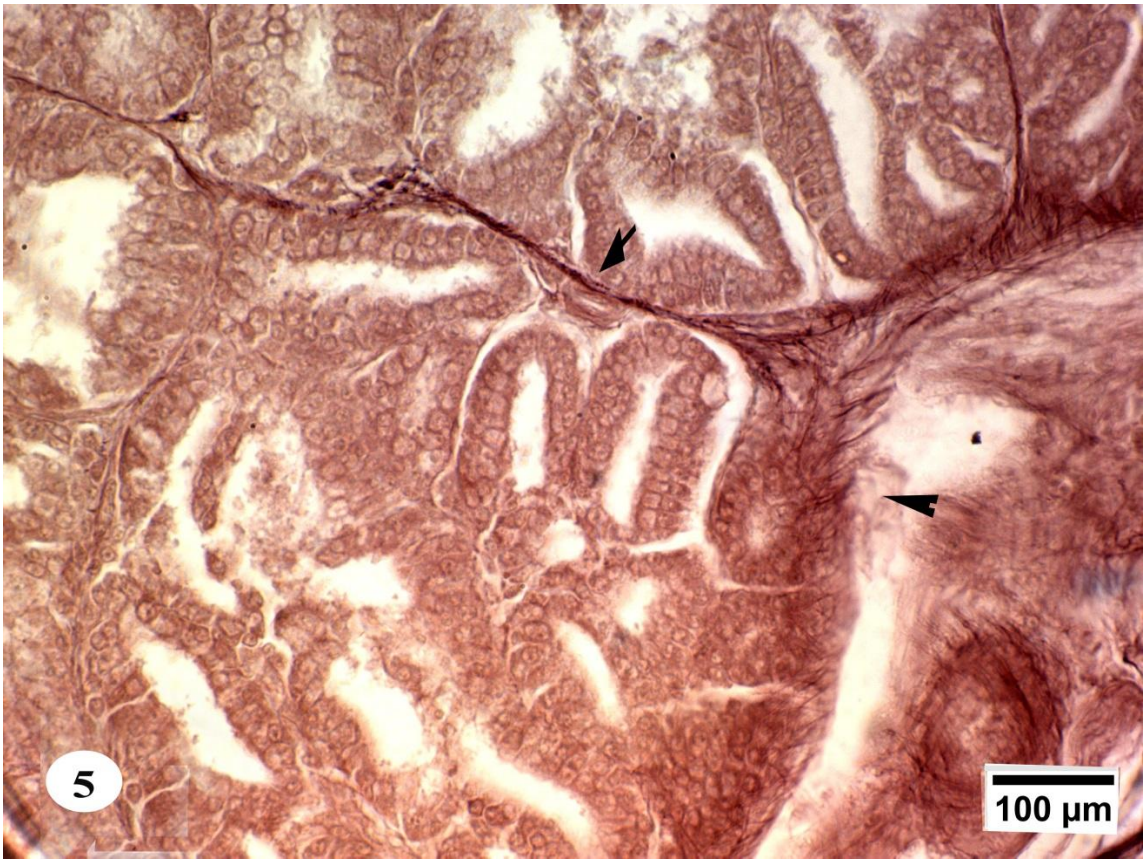
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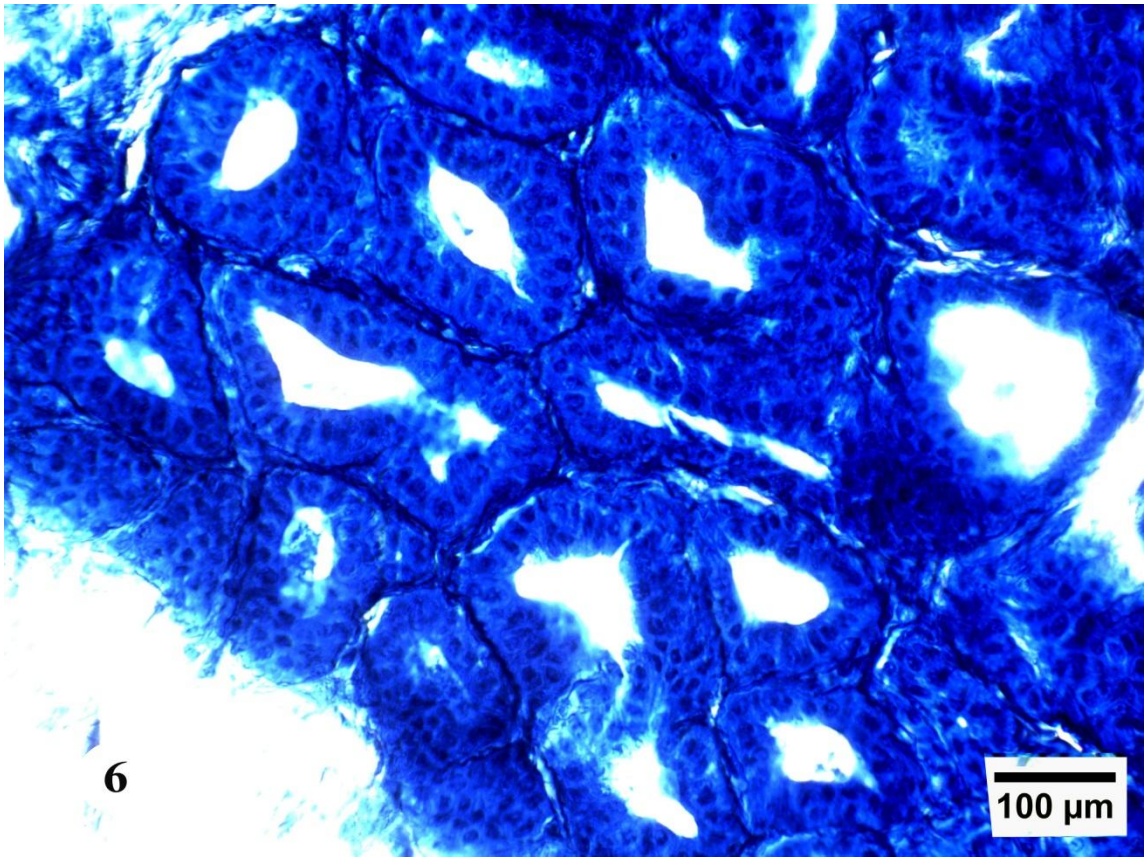
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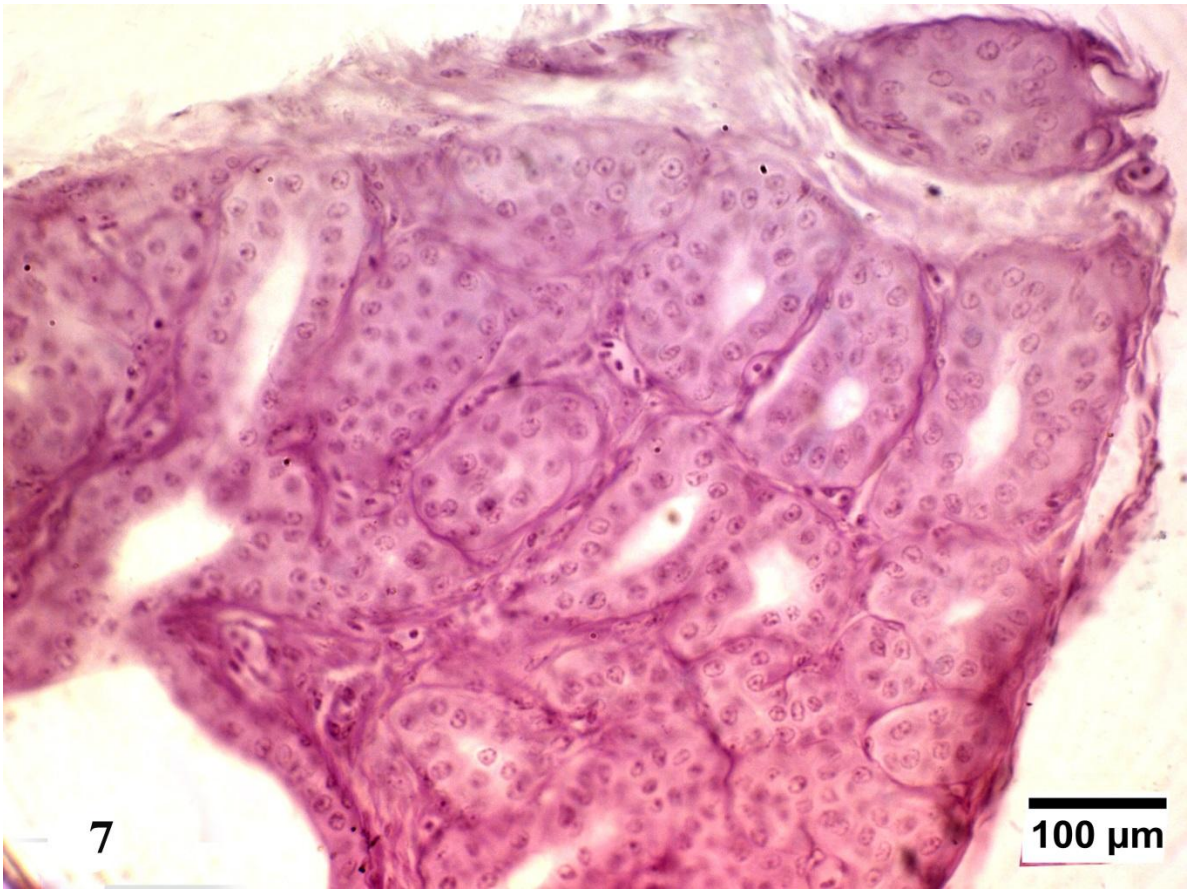
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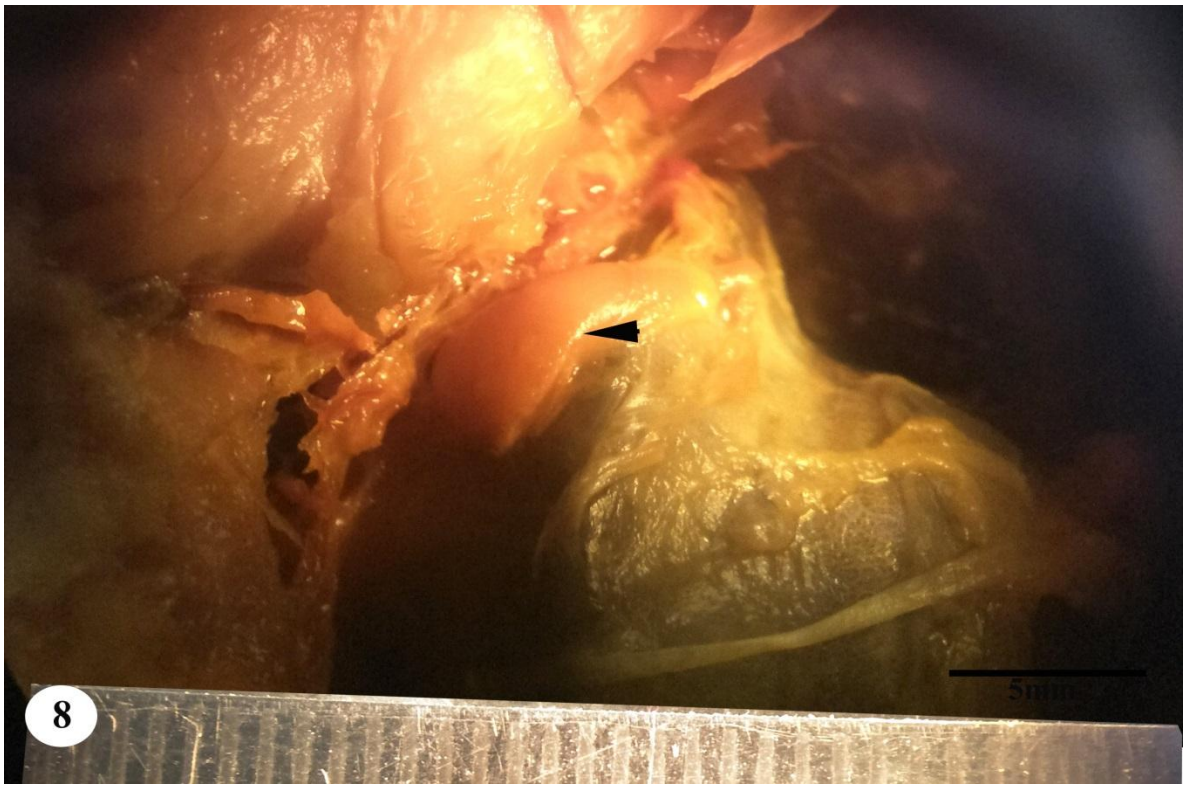
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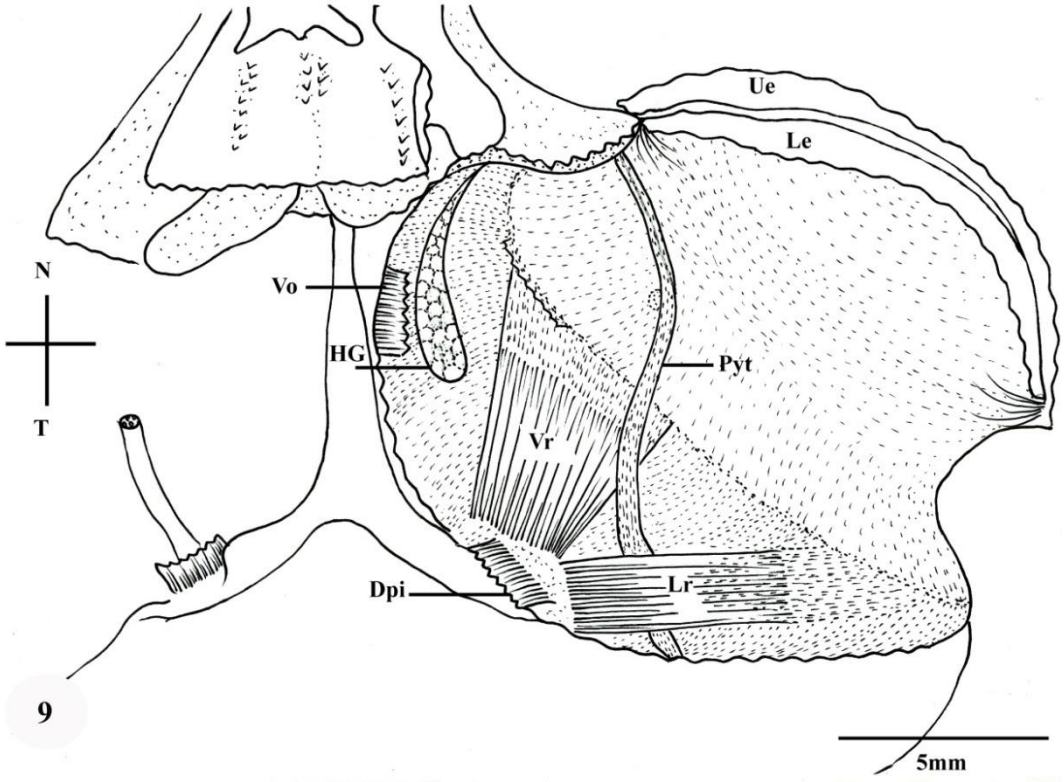
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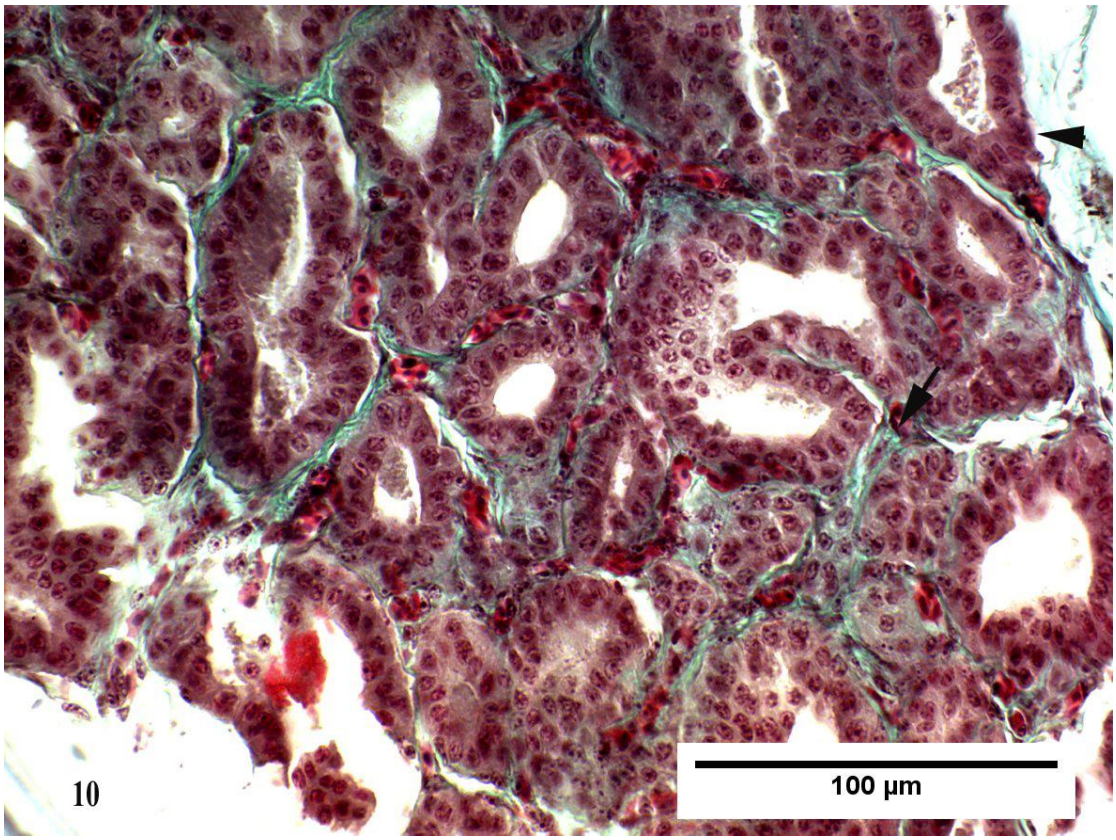
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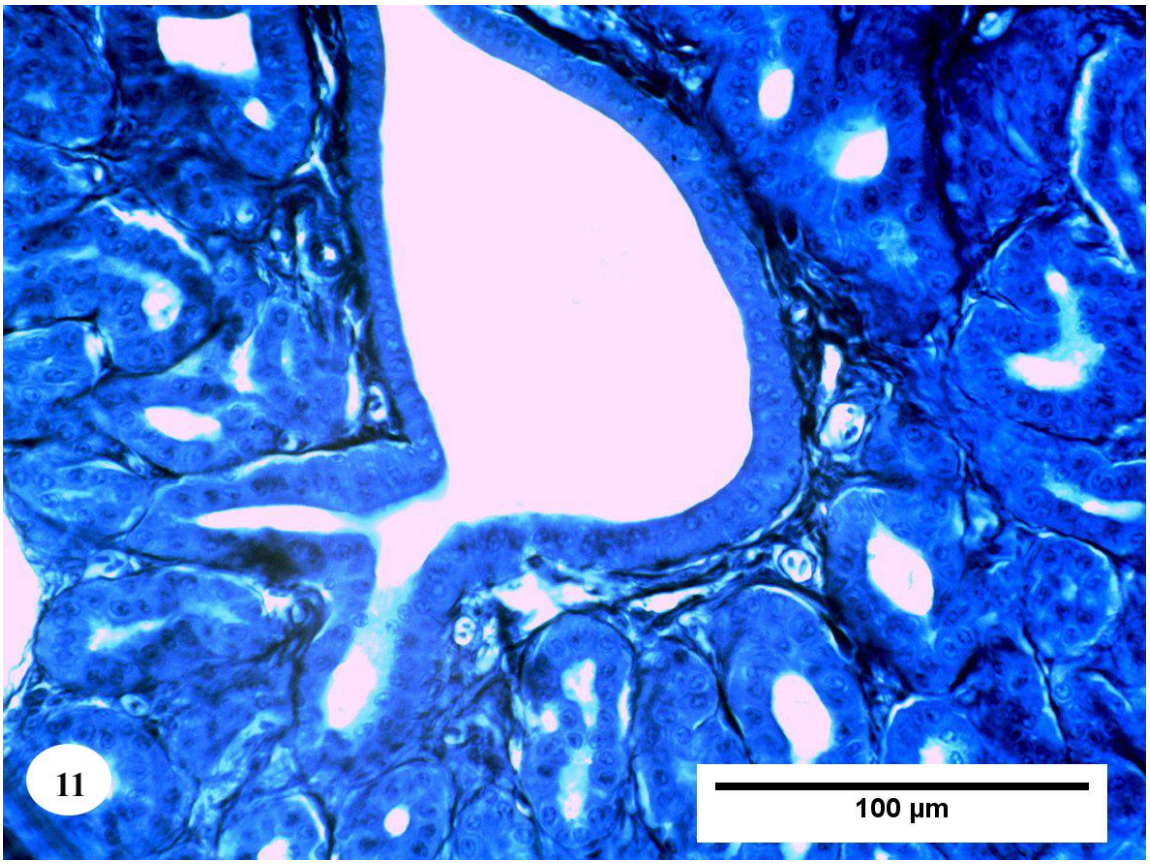
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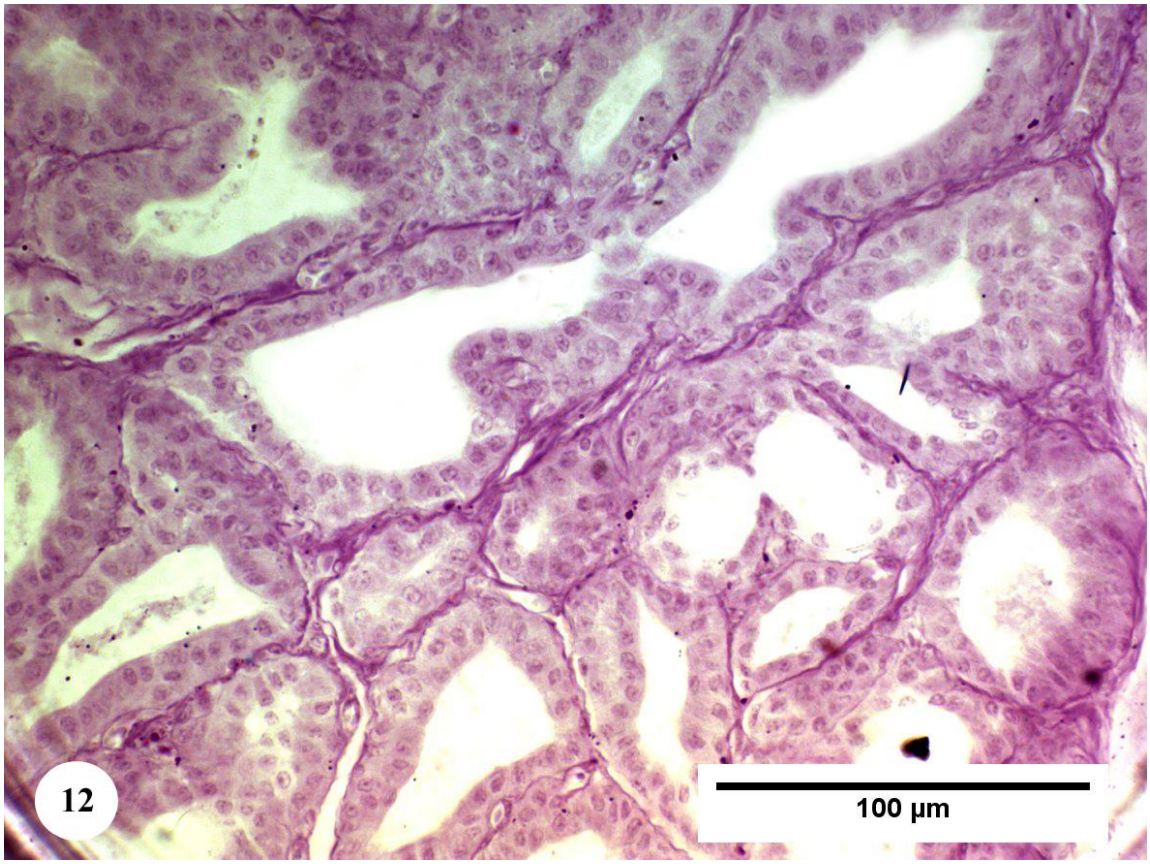
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