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RESEARCH ARTICLE

Histochemical and Histological Investigations on Duvernoy's Gland in *Natrix tessellata* (Squamata: Colubridae) [1]

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Summary

Duvernoy's gland, an oral gland located in temporal region, is only found in Colubrid snakes. This is the first report describing morphological and histological structure of Duvernoy's gland in *Natrix tessellata* (Laurenti, 1768). It is surrounded by a connective tissue layer which penetrates into the gland by forming many septa, dividing the glandular body into lobules and including the acini and the inner ducts. Duvernoy's gland is formed by seromucous acini composed of seromucous cells, and mucous acini composed of mucous cells and they are recognized by their histochemical characteristics. The gland is mainly organized in seromucous acini, mucous cells are restricted to the gland center region and to the inner secretion duct lining epithelium.

Keywords: *Natrix tessellata, Colubridae, Duvernoy's gland, Seromucous acini, Mucous acini*

Natrix tessellata'nın (Squamata: Colubridae) Duvernoy Bezinde Histolojik ve Histokimyasal İncelemeler

Özet

Temporal bölgede konumlanan ve oral bir bez olan Duvernoy bezi sadece colubrid yılanlarda bulunur. Bu çalışma *Natrix tessellata* (Laurenti, 1768)'da Duvernoy beziyle ilgili ilk morfolojik ve histolojik çalışmadır. Bez bağ dokusu tarafından kuşatılır. Bağ dokusu bezin iç kısımlarına uzanarak asinuslar ve iç salgı kanallarını da kuşatarak lobulleri oluşturur. Duvernoy bezi seromukoz hücrelerden oluşan seromukoz asinuslar ve mukoz hücrelerden oluşan mukoz asinuslardan oluşur. Asinuslar histokimyasal özelliklerine göre karakterize edilirler. Bez temel olarak seromukoz asinuslardan oluşurken, mukoz hücreler bezin merkezi ve iç salgı kanalı etrafı ile sınırlı kalır.

Anahtar sözcükler: *Natrix tessellata, Colubridae, Duvernoy Bezi, Seromukoz asinuslar, Mukoz asinuslar*

INTRODUCTION

The oral glands, which are varies in reptiles, are located in mouth cavity and its periphery; opposite and beneath of tongue, throughout upper and lower lips, nearby of nasal cavity or at temporal region and their secretions are directed into mouth cavity. Among the reptiles, especially snakes are equipped with oral glands to immobilize the prey and lubricate the food [1-3]. In snakes infralabial, supralabial glands, venom gland and Duvernoy's or parotid glands are the most well-known oral glands [4].

The groups having real venom delivery system among contemporary reptiles are venomous snakes (Colubridae, Atractaspididae, Viperidae, Elapidae, Crotalidae) and helodermatid lizards (*Heloderma suspectum* and *Heloderma horridum*) [2,5,6]. Mandibular gland specialized as a venom gland in helodermatid lizards extends along lower jaw. Venom is directed to mandibular teeth with many channels (*Heloderma suspectum*) or with a single channel (*Heloderma horridum*) origined from the venom gland. In venomuos snakes, all the structures of venom system (gland, muscles, fang etc.) are located along the upper jaw [2,7-9].

Real venom gland and Duvernoy's gland are only exist in species belonging Colubroidea superfamily [10]. In fact,

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both Duvernoy's gland and real venom gland are of venom gland characteristics. Despite both types of glands are considered to be homologous [1], morphological and functional differences are available. Unlike Duvernoy's gland, real venom gland consists of actual and accesory venom gland. These two parts are connected with primary channel. These parts are absent in Duvernoy's gland and its secretion is carried with primary channel to fang groove. While real venom gland is available in species of Elapidae, Viperidae and Atractaspididae families, Duvernoy's gland is present at some species of Colubridae family (30-40% of Colubrid snakes) [1,7,11-13].

Information on function of Duvernoy's gland is very rare [1,9]. It is unusual that Duvernoy's gland empties secretions to kill prey rapidly like a real venom gland [1,14,15]. It is caused by the differences in excretion mechanisms of real venom gland and Duvernoy's gland. In real venom gland, jaw adductors derived from the skeletal muscle has been included in gland capsule. Contraction of these compressor muscles are directly effective on the main venom gland. According to this hypothesis, increase of pressure as a result of the effect of compressor muscles on the gland give rise to leak of venom in pressure. In Duvernoy's gland, jaw muscles or the ones derived from these muscles are scarcely included in gland capsule. Generally, jaw muscles pass medially without participating to gland capsule. Therefore Duvernoy's gland secretion flows as leakage [1].

Natrix tessellata is distributed all over the Turkey, Asia and Europe. Although it is abundant, there is no any histological study on Duvernoy's gland in *N.tessellata*. Aim of this study was to investigate the morphological and histological properties of Duvernoy's gland in *N. tessellata* and to prove histologically that it is a venomous species.

MATERIAL and METHODS

Animals

The protocol was approved by the animal ethic committee of Ege University, Faculty of Medicine (2009-134). Three adult individuals of *Natrix tessellata* were obtained from the edges of Beyşehir Lake (between 37° 45' North latitude and 31° 36' East longitude) and were brought alive to the laboratory. Following anesthesing with ether, Duvernoy's gland of the snakes was dissected *(Fig. 1)* and processed for light microscopic studies.

Histology and Histochemistry

The glands were fixed in Bouin's fixative for 24 h, dehydrated in ethanol and put to xylol for transperancy, embedded in paraffin. Paraffin blocks were sectioned serially in 5 µm thickness using microtome (Baird & Tatlock). These sections were stained with Mayer's Haematoxylin-Eosin (H&E), Periodic Acid Schiff (PAS), combined PAS and Alcian-Blue pH 2.5 and Mallory's Trichrom. They were examined by light microscopy and then photographed with Olympus CX31-Altra 20 Soft Imaging System.

Fig 1. Dissection of Duvernoy's gland in *Natrix tessellata*
Şekil 1. *Natrix tessellata*'da Duvernoy bezinin çıkartılması

RESULTS

Duvernoy's gland is thin and elongated shaped and consists of many small lobules. It measures around 8 mm long, and 2 mm wide *(Fig. 2)*.

Fig 2. Morphological view of Duvernoy's gland
Şekil 2. Duvernoy bezinin morfolojik görünümü

Duvernoy's gland is surrounded with a connective tissue capsule. It dissects gland into lobules. Duvernoy's gland is mainly composed of mucous and seromucous acini. The acini are formed epithelium arranged as a single layer

constituted of mucous and seromucous cells, recognized by their histochemical properties. Seromucous acini are more intensive *(Fig. 3)* and surround mucous acini *(Fig. 4)*.

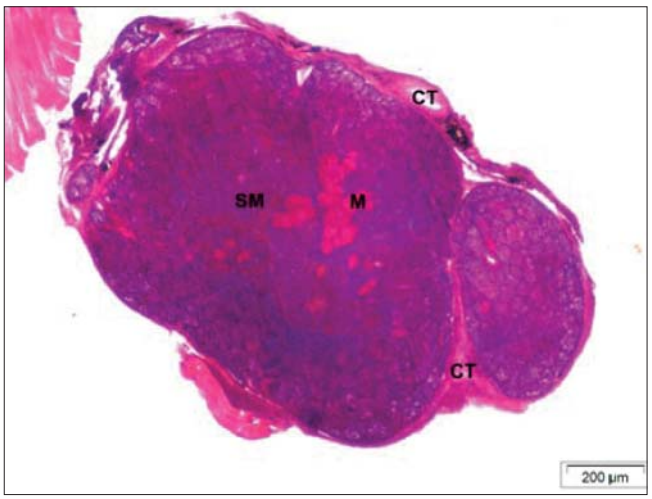

Fig 3. Histological view of Duvernoy's gland. Mucous (**M**) and seromucous acini (**SM**) and connective tissue (**CT**) in the form of many septa, H&E

Şekil 3. Duvernoy bezinin genel görünümü. Mukoz (**M**) ve seromukoz asinuslardan (**SM**) oluşan bez dışarıdan bağ dokusundan (**CT**) bir kapsülle kuşatılmıştır, H&E

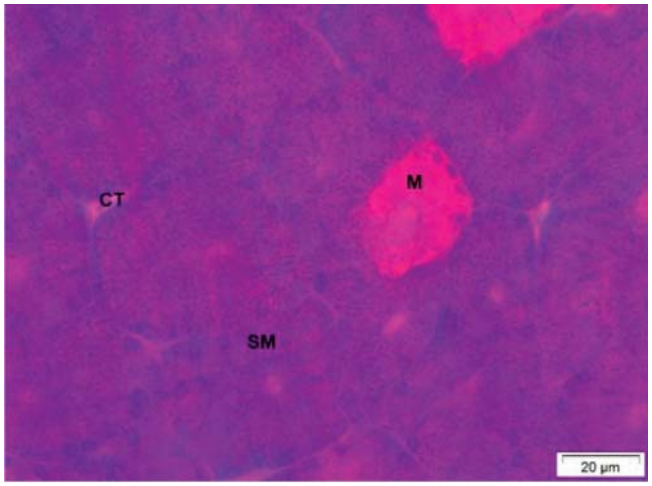

Fig 4. A large quantity of seromucous acini (**SM**) encircled mucous acini (**M**). **CT:** Connective tissue, H&E

Şekil 4. Mukoz asinusların (**M**) etrafını kuşatan yoğun orandaki seromukoz asinusları (**SM**). **CT:** Bağ doku, H&E

In Haemotoxylin-Eosin staining method, mucous acini are light pink, but seromucous acini are dark purple. While mucous cells are characterized with flat basal nuclei, seromucous cells have oval basal nuclei. In addition, lumina of mucous acini is larger than seromucous acini *(Fig. 5)*.

Inner duct are lined by cylindirical mucous cells. Seromucous acini were seen in the periphery of mucous cells (*Figs. 6* and *7*).

Periodic-Acid Schiff (PAS) histochemical method showed that mucous acini were stained dark purple but seromucous acini were stained light purple. Due to the presence of mucin, mucous cells give a strong reaction with PAS *(Fig. 8)*.

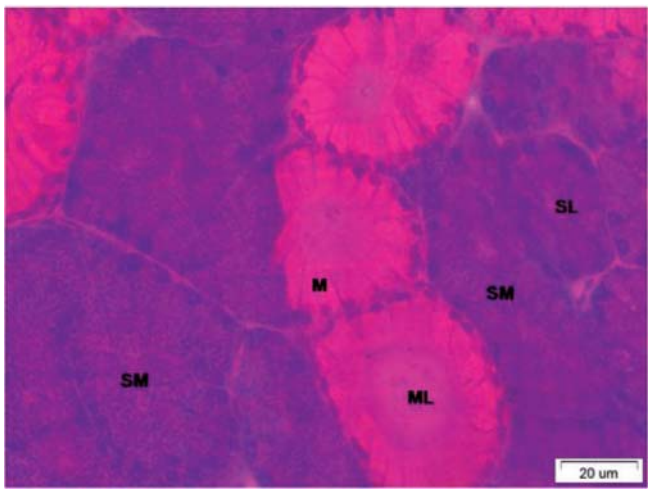

Fig 5. M: Mucous acini, **ML:** Mucous acinus lumina, **SM:** Seromucous acini, **SL:** Seromucous acinus lumina, H&E

Şekil 5. M: Mukoz asinuslar, **ML:** Mukoz asinus lümeni, **SM:** Seromukoz asinuslar, **SL:** Seromukoz asinus lümeni, H&E

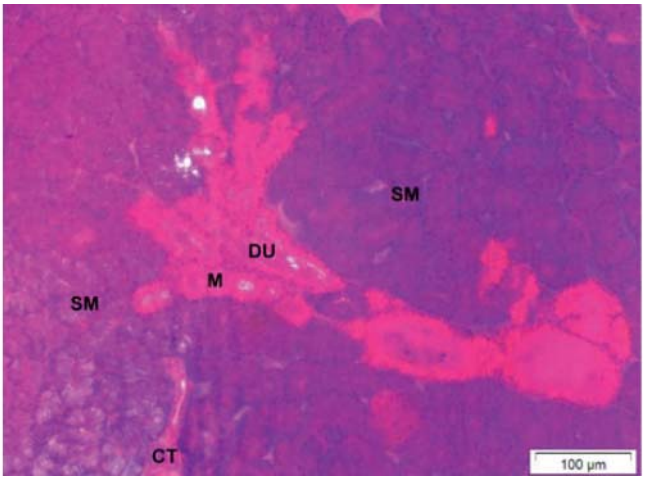

Fig 6. DU: Duct, **SM:** Seromucous acini, and **M:** Mucous acini, **CT:** Connective tissue, H&E

Şekil 6. DU: Salgı kanalı, **SM:** Seromukoz asinuslar ve **M:** Mukoz hücreler, **CT:** Bağ doku, H&E

As a result of staining with Mallory's trichrome, mucous cells is easily distunguished from seromucous cells by staining light orange due to mucous and having flat basal nuclei *(Fig. 9)*.

Another histochemical method is Alcian Blue + Periodic Acid Schiff conjugation. Alcian blue gave weak reactions with the seromucous cells, so they stained light blue, mucous cells reacted with PAS staining pink *(Fig. 10)*.

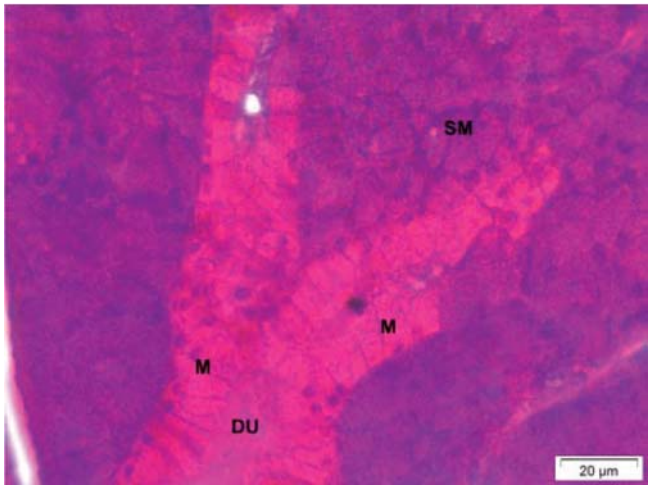

Fig 7. The mucous cells (**M**) surrounding around duct (**DU**). **SM:** Seromucous cells, H&E

Şekil 7. Salgı kanalının (**DU**) etrafını çevreleyen mukoz hücreler (**M**), **SM:** Seromukoz asinuslar, H&E

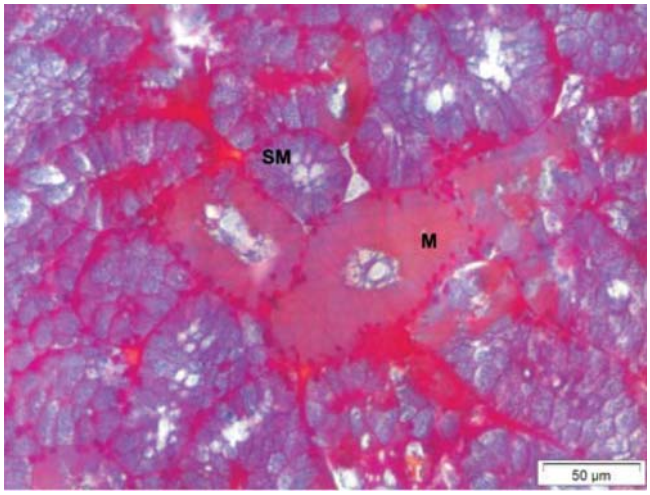

Fig 9. Two mucous acini (**M**) are easily seen light orange with basal flat nuclei. Mallory's Trichrom

Şekil 9. İki mukoz asinus (**M**) açık portakal renkte boyanmaları ve yassı nukleusları ile kolayca ayırt edilirler. Mallory's Trichrom

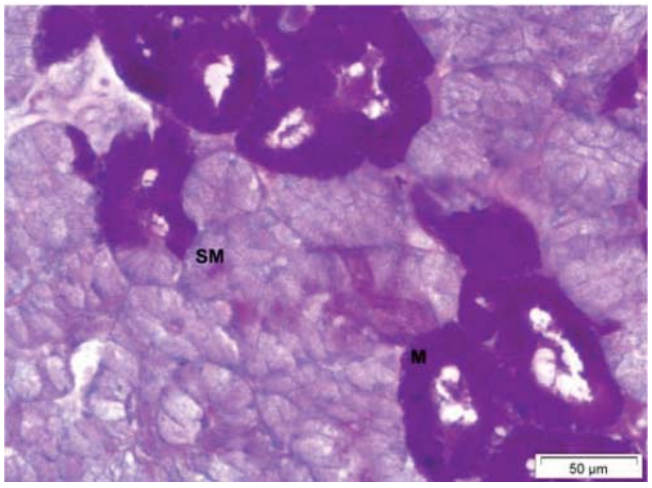

Fig 8. Weakly stained seromucous cells (**SM**); Mucous cells (**M**) with dark colour and large lumina, PAS

Şekil 8. Seromukoz hücreler (**SM**) daha açık renkli, Mukoz hücreler (**M**) koyu renkli ve geniş lümenlidir, PAS

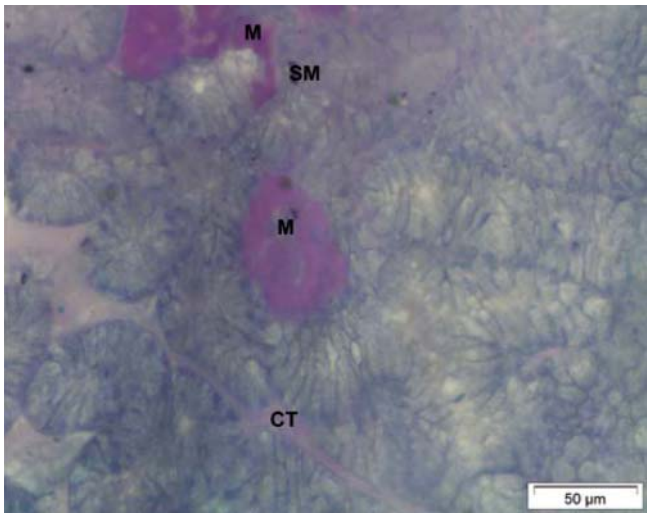

Fig 10. CT: Connective tissue, **SM**: Seromucous acini, **M:** Mucous acini, Alcian Blue + PAS

Şekil 10. CT: Bağ doku , **SM:** Seromukoz asinuslar, **M:** Mukoz asinuslar, Alcian Blue + PAS

DISCUSSION

Although oral glands (supralabial, infralabial, Duvernoy's gland and venom gland etc.) show diversity and are widely exist in snakes, information about morphology and function of them is very scarce. In some papers, it is cited only morphology of glands, but there is not any functional and phylogenetical peculiarities [16].

Both infralabial and supralabial glands have a similar function in terms of lubrication of prey during ingestion by secreting mucous [2]. However, infralabial glands may have different functions. For example in Colubroids, a group of dipsadine snakes named "goo-eaters" have advanced infralabial glands specialized to produce mainly serous secretion in nature. In addition, Duvernoy's gland of these snakes are not developed like in other Colubrids [10]. So, infralabial glands in dipsadine snakes undertake similar function of Duvernoy's gland. Consequently infralabial glands of dipsadine snakes have same histological structure in Duvernoy's gland having both seromucous and mucous cells.

Oral glands in some dipsadine goo eaters snakes were investigated by Oliveira et al.[10]. *Dipsas indica*'s infralabial gland has more mucous cells when compared to Duvernoy's gland of *N. tessellata*. On the other hand seromucous acini are more intensive in infralabial gland of *Sibynomorphus mikanii* and *Atractus reticulatus* like in the *N. tessellata*'s Duvernoy's gland.

In view of these results, it is suggested that secretion of *D. indica*'s infralabial gland is less toxic than *N. tesellata*'s secretion. For this reason, there are some advices to increase of toxicological studies related this species in question. According to our results, mucous acini are usually limited in the center of the gland and around the internal secretion duct. A large quantity of gland is composed of seromucous acini. In *Dipsas albifrons*, mucous acini are only present in the anterior region of gland [17]. Duvernoy's gland in *N. tessellata* shows that a large portion of the mucous cells are limited around the inner duct like in *Dipsas albifrons* and *Sibynomorphus mikani*. In view of these data, it can be said that secretion of three species in question have toxic effects on the prey.

Periodic acid schiff (PAS) method on Duvernoy's gland of *Natrix tessellata* indicated that mucous acini showed strong positive reaction and stained in dark purple. Same was observed in *Atractus reticulatus* [10]. Mucous and seromucous cells are observed in same acinus in the Duvernoy's gland of *Atractus reticulatus* [10], *Atractus zebrinus* [17], *Micrurus corallinus* [18] and *Philodryas patagoniensis* [19]. However, this is not the case in *Natrix tessellata*.

It is suggested that myoepithelial cells are closely related with secretory cells using transmission electron microscope in *Rhabdophis tigrinus* [20]. In these studies, it is understood that duct epithelium comprise typical mucous secreting cells and there are low density of electron granules in the cytoplasm of these cells. This case is accordance with our results in that inner ducts comprise mucous cells in Duvernoy's gland of *Natrix tessellata*.

Recently, it is understood that venom of colubrid snakes has an toxic effect on prey's nervous system (neurotoxic). The LD_{50} value of *Natrix tessellata* on the mouse was determined as 25.0 (mg/kg). Venom has activities such as arginine esterase, acid phosphatase, alkaline phosphatase, protease and fosfodieasterase [21]. Our results showed that Duvernoy's gland was composed of mainly seromucous cells and supported histologically the view related to be a venomous species of *Natrix tessellata* which was accepted as venomous one in last years. Because of the covering of Duvernoy's gland inner duct with mucous cells and location of fang in behind of maxillary bond, we cannot say that it has a potential danger for human health. But we believe that more detailed works should be performed to reveal medicinal and pharmacological importance of Duvernoy's gland secretion.

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