PECULIARITIES OF THE MORPHOHISTOLOGICAL STRUCTURE OF THE DUVERNOIS'S IN THE COLUBER KARELINI SNAKES

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Abstract. This article presents the morphohistological features of the structure of the Duvernois glands in snakes of the *Coluber karelini* species. It is noted that narrow-shaped snakes, to which the snake of the Coludridae family belongs, belongs to the anteroscopic. According to the morphological structure, the glands are exocrine, which allows this snake to excrete poison directly into the victim, moving along the main duct. Despite a sufficient number of works by both foreign and domestic authors, the question remains open. The results are presented, the corresponding conclusions are drawn.

Key words: Duvernois glands, *Coluber karelini* snakes, merocrine type of secretion, poisonous apparatus.

Relevance. The Colubridae family includes the *Coluber ravergieri, Coluber karelini, Rhabdophis tigrina*, and *Coronella austriaca* [1].

In the process of evolution, in the digestive system of snakes, special adaptations have been developed for swallowing large prey and a poisonous apparatus has formed, which ensures its immobilization. Swallowing the whole prey required significant rearrangements in the skull, and especially in the jaw apparatus: the lower jaws can deviate from the upper ones almost at right angles; in addition, ligaments that allow each half of the jaw to move away from one another interconnect them. Due to this, the snake is able to swallow prey, the diameter of which exceeds the diameter of the head of the snake itself [2].

Evolutionary transformations in the venomous apparatus of snakes from various families reflect the main features of their feeding. The natural toxicity of the saliva of individual representatives of snakes is explainable in terms of the presence of various digestive enzymes in it. This property was consolidated in the process of evolution, as it increased the efficiency of hunting. Gradually, the salivary glands - upper lip, temporal - began to specialize in the production of a predominantly poisonous secretion. At the same time, the formation of an apparatus for the active injection of poison into the victim's body took place [3]. Individual teeth, located at the posterior or anterior end of the upper jaw, increased in size, a groove appeared on their front surface, along which poison dripped. Then, when the groove was closed, an internal channel was formed, which opens with an outlet near the apex of the tooth, which significantly increased the efficiency of introducing the poison into the victim's body [4-5]. In already-shaped snakes, venomous teeth sit on the posterior edge of the maxillary bone and are separated from the others by an edentulous gap; therefore, they are usually called posterior-furrowed [6]. In other venomous snakes, venomous teeth are located on the anterior edge of the maxillary bone; they are referred to as anteroscopic snakes.

The Colubridae family unites over 60% of all snake species. The subfamily of true snakes (Colubrinae) includes the vast majority of all other snakes. Among them, there are species whose saliva has a toxic effect: the multicolored snake (*Coluber ravergieri*), the tiger snake (*Rhabdophis tigrina*), and the common copperhead (*Coronella austriaca*). Another subfamily - false snakes (Boiginae), or suspiciously poisonous, includes species that have a poisonous gland (Duvernois gland), the ducts of which end at the base of the poisonous teeth. Since the teeth are located deep in the mouth at the posterior edge of the maxillary bone, the snake can only bite the victim in the mouth [7-8]. In this regard, the procedure for obtaining venom from posteroscopic snakes presents certain difficulties. To do this, suction of poison from the base of a poisonous tooth is used, including with the use of microaspiration technique [9, 10, 11].

Poisonous glands are located behind the eyes, have an alveolar structure, and in some representatives, for example, *Boiga trigonatum*, *Telescopus fallax*, they reach large sizes.

At the same time, there are currently no data on the histological characteristics of the venomous apparatus of Duvernois in snakes of the genus *Coluber karelini*, which served as the relevance for the study.

Purpose of the study. To study the histological structure of Duvernois glands in snakes of the *Coluber karelini* species.

Materials and methods. To achieve this goal, the results of the histological structure of the Duvernois glands of 10 specimens of the *Coluber karelini* snake was studied.

The animals were killed by decapitation. Immediately after decapitation, the glands were dissected.

For histological studies, conventional techniques were used. A 10% neutral formalin solution was used as the fixing liquid. The fixation lasted 4 days. After fixation, the objects were washed under running water for one day.

Then the wiring was carried out according to the standard scheme, which included dehydration in alcohols of increasing concentration, embedding in paraffin, obtaining sections on a MZ-2 microtome, sections 4-5 microns thick were stained with hematoxylin and eosin.

Statistica Windovs 7.0 software was used for statistical processing of the results.

Results and Discussion. The gland consists of many alveolar parts and is actively involved in the biosynthesis of toxic components. In snakes, venom appears most often when it is active and well fed. Snake poisoning depends on the size of the snake's body as well as the size of the Duvernois glands.

The seasonal increase in snake poisoning occurs mainly during the summer months when snakes are well fed. An outbreak of poisoning is observed in the period from September to October and the lowest in January-March. From September to October, the rhythms of life of snakes decrease and the intensity of feeding begins, they prepare for winter. From April to May, cases of poisoning begin to increase.

Snake poisoning is one of the most important areas of zoology today, which requires study with a detailed description of the composition and mechanism of action of snake venom.

The histological Duvernois glands can be subdivided into the posterior (main) part, represented by multi-branched serous tubules, the main duct and the anterior part, consisting of mucous tubules. The anterior part of the venom gland of the *Coluber karelini* snake is represented by tubules with wide lumens covered with mucous epithelium (Fig. 1).

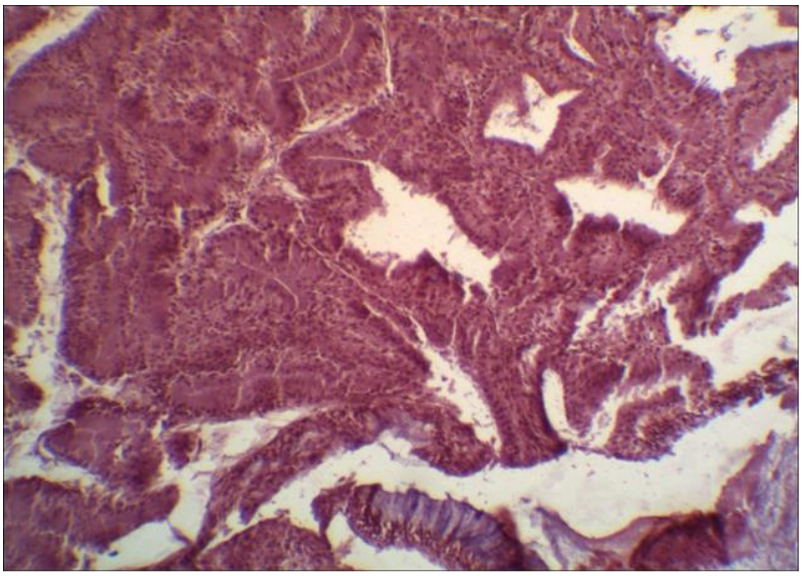

Fig. 1. End tubular secretory sections and excretory duct. Staining with hematoxylin and eosin. Magnified 7X20.

A connective capsule rich in collagen fibers, nerve endings and spindle-shaped cell elements (fibroblasts) surround the Duvernois poison gland on all sides. In the section, iron has a lobular structure. Connective tissue septa extending from the capsule into the interior of the gland isolate its lobules from each other.

Loose connective tissue containing blood capillaries of various sizes is located between the secret-producing complexes of the gland. The parenchyma of the posterior part of the gland is represented by secretory tubules covered with the same type of highly prismatic cells that exhibit visible secretory activity (Fig. 2,3). There are numerous small ducts (Fig. 4).

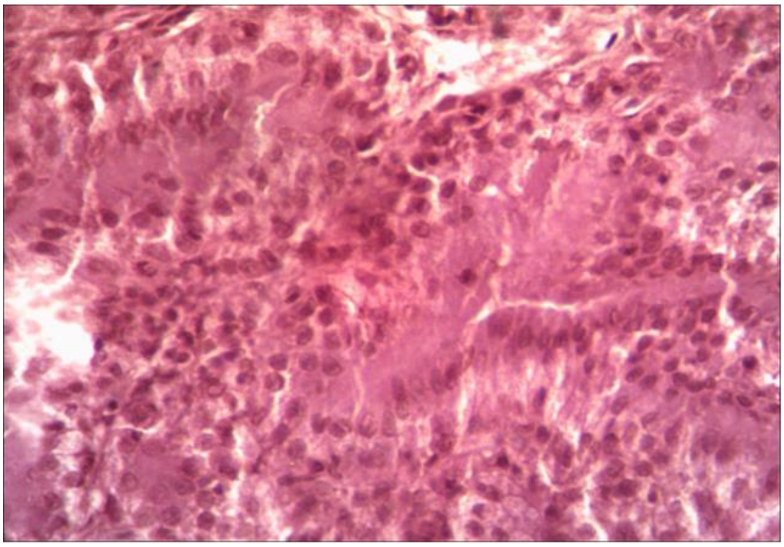

Fig. 2. Terminal secretory sections (serous-colonic cells). Staining with hematoxylin and eosin. Magnified 7X20.

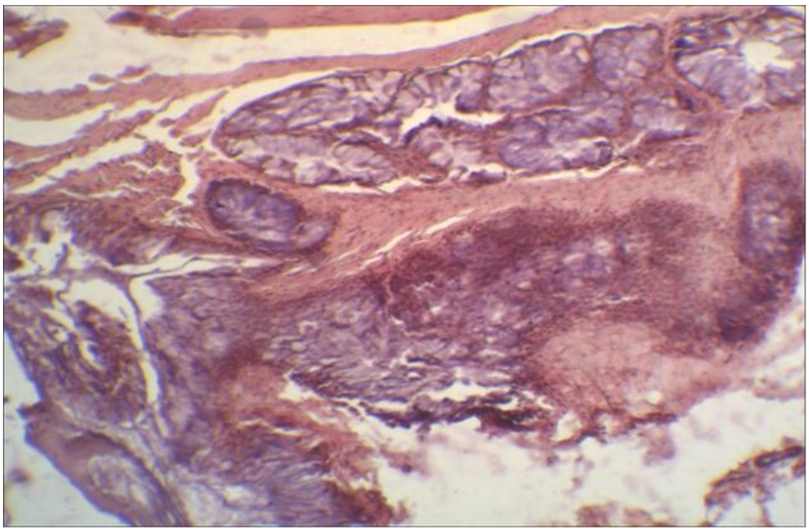

Figure: 3. Terminal secretory cells (mucinous goblet cells). Staining with hematoxylin and eosin. Magnified 20X7.

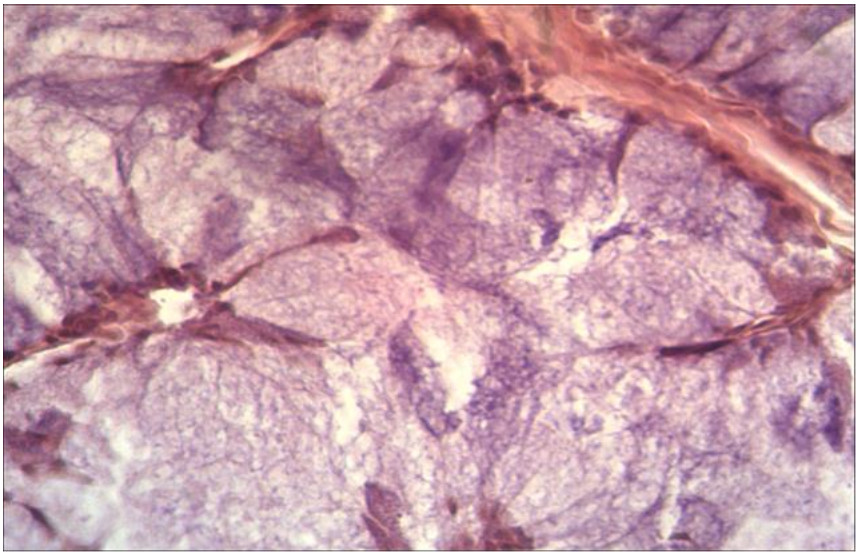

Figure: 4. Excretory ducts of the mucinous section of the Duvernois gland. Stained with hematoxylin and eosin. Magnified 20X7.

The cells lie on a poorly expressed basement membrane and have a pronounced polarity, which is due to the direction of secretory processes from the basal part to the apical part.

Follicle-like structures, various shapes and sizes represent the serous part. At low magnification, the lobular structure is clearly visible (Fig. 5). Thin layers of connective tissue separate the cavities; the cavities are lined with one layer of cubic epithelium. The content of the cavities is dark pink and depends on the protein content in the secret (Fig. 6).

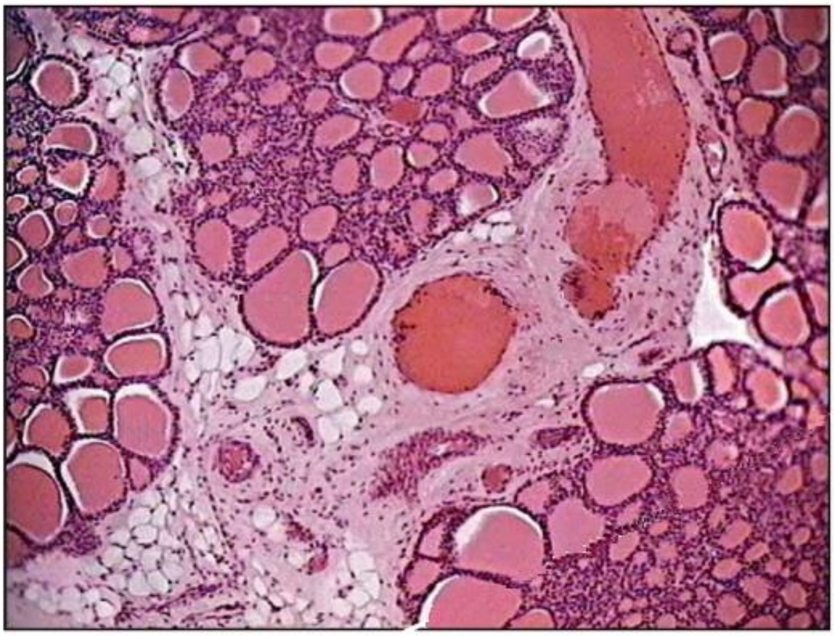

Fig. 5. Anterior part of the Duvernois gland. Staining with hematoxylin and eosin. Magnified 20X7.

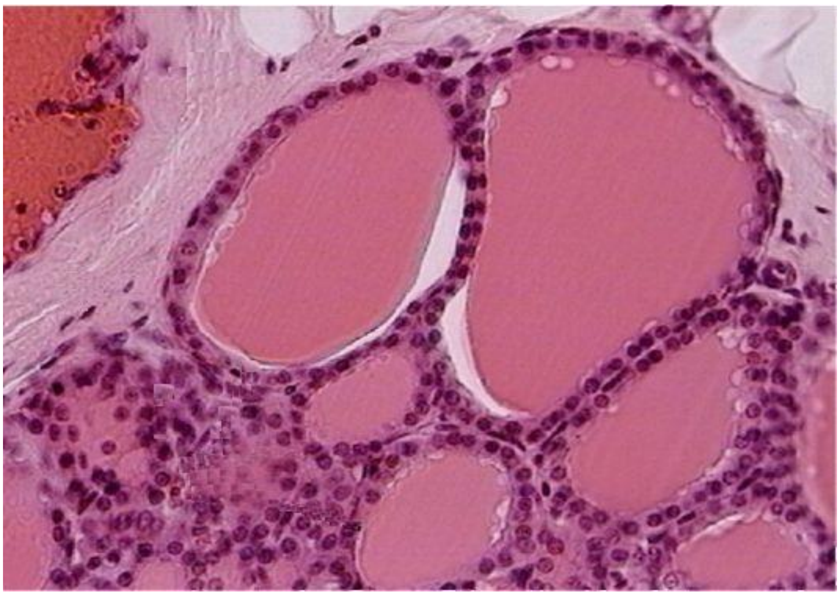

Figure: 6. Serous duvernoy gland. Staining with hematoxylin and eosin. Magnified 7X20.

Mucous cells are larger and lighter in color than serous cells. Mucous cells lie on a poorly defined basement membrane, like serous cells, and have a pronounced polarity. In the basal part of the cells there is a large nucleolus containing one or two nucleoli. The intracavitary substance of the end secretory sections of the anterior part of the gland looks like a homogeneous, poorly stained light mass.

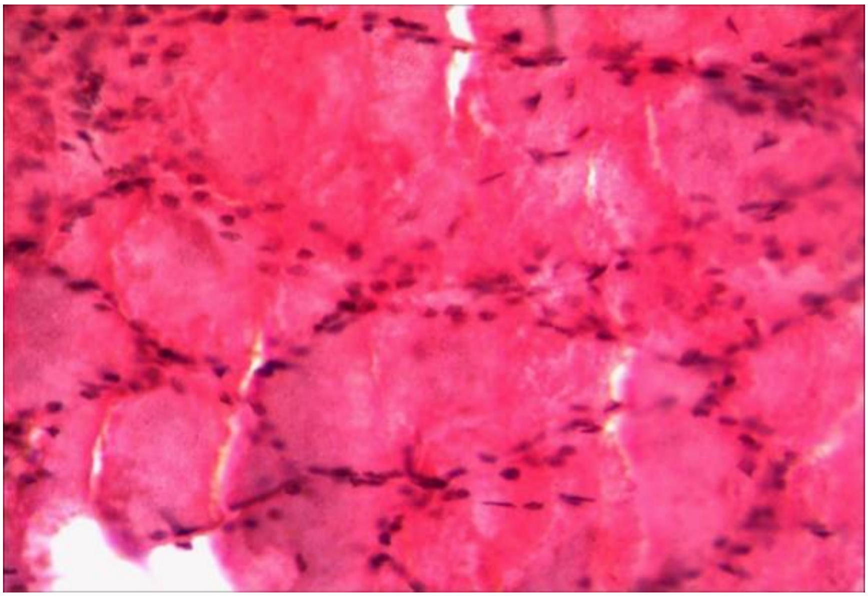

Figure: 7. Terminal alveolar secretory sections (serous cells) of a snake of the Coluber karelini species. Staining with hematoxylin and eosin. Magnified 20X7.

In the basal area of cells, there is a large nucleus containing one or two nucleoli. When the secretory cells of the posterior region are stained with hematoxylin and eosin, basophile of their apical parts and a slight acidophilus of the supranuclear region are observed.

The intracavitary substance of the end secretory sections of the posterior part of the gland looks like a homogeneous acidophilic mass. Sometimes cells of the desquamated epithelium and individual nuclei are distinguished in it. The poison accumulates in the lumen of the secretory tubules. The poison that does not fit into the tubules enters the wide main duct, where the bulk of the poison is deposited. The main duct is a thick-walled circular tube covered from the inside with a single-layer cubic epithelium (Fig. 8).

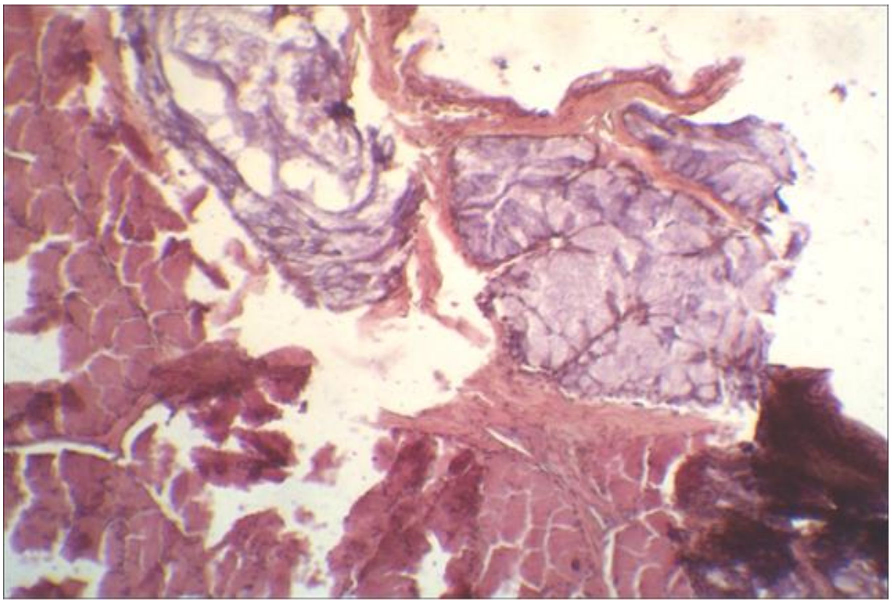

Figure: 8. The main duct of the gland. Staining with hematoxylin and eosin. Magnified 20X7.

At the same time, according to Sh.I. Kamolova as a result of the conducted studies of the ecological and biological characteristics of the water snake and the red-striped snake, we have specified and corrected the data on their occurrence in biogeocenoses of Uzbekistan; seasonal and daily activity, the nature of nutrition and reproduction.

Our observations showed that the active period of life of the water snake and the red-striped snake lasts for nine months. Snakes are cold-blooded organisms, so their activity is highly dependent on the temperature of the external environment. With a decrease in temperature, snakes become inactive, fall into a daze or in a state of hibernation, in which the level of free fatty acids, glucose and lactate in the blood serum significantly decreases, which indicates a significant restructuring of lipid and carbohydrate metabolism. At the same time, under conditions of cold numbness, a significant suppression of the basal metabolism is noted.

Small fish, frogs, toads and tadpoles, less often chicks and small mammals serve as food for the water snake. The red-striped snake feeds mainly on small vertebrates, rodents, birds, foot-mouths, which it eats alive, however, it preliminarily kills larger prey with the help of poisonous teeth.

Among the snakes in the fauna of Uzbekistan, there are no species that pose a potential danger to humans, which is mainly determined by the structural features of their venomous apparatus. There is evidence that the secret of their glands is poisonous enough for warm-blooded animals, since with the help of their venom, snakes can kill or render harmless their prey. However, the location of the grooved teeth at the posterior end of the long upper jaw creates unfavorable conditions for wounding with these teeth and, therefore, for introducing poison into the wound. In addition, some of the juvenile snakes only bite in exceptional cases. The combination of these features makes many of the snakes practically harmless to humans.

Also under the data of M.E. Abubakirova the arrow-snake is widespread in fixed sands, saline and clay deserts, loess hills, irrigated lands and tugai. We caught 22 snake arrows in various districts of the Surkhandarya region (Termez, Angor, Sherabad districts). The size of the reptiles was 450-1002 mm.

Rodent pores and piles of stones are characteristic hiding places for these species of snakes, and they can be found in the holes of the gray monitor lizard. The main food of the arrow-snake is various lizards: Transcaspian and sandy roundheads ruled and reticulated lizards. Young snakes feed on insects.

Mating at the arrow-snake in the conditions of the Surkhandarya region begins in late March - early April, but individual mating snakes are found until the end of May. Newborns measured 210-280 mm.

Arrows-snakes with a sign of molting and spilling individuals were found at the end of March. Fresh creepers were found in Sherabad region at the end of April, in October and mid-November. In captivity, molting of this reptile species occurs in all seasons. Thus, it can be assumed that the arrow-snake molt occurs 3-4 times a year.

Multicolored snake (*Coluber ravergieri*). It is one of the most widespread species of snakes in the Surkhandarya region. The ruins, abandoned areas serve as shelters for the multi-colored snake; this reptile is found in. abandoned buildings in big cities, in cattle houses and even in residential buildings.

In March, the multicolored snake was seen from 10 to 12 hours, in April - in the morning from 8 to 12 hours and in the afternoon - from 12 20 above until 20 40 minutes. In May-July, snakes are most active in the morning and evening, sometimes they can be found at night. In September, just like in early spring, snakes are found at noon from 10-14 hours. In October, they start looking for hibernation sites. An autopsy of 10 stomachs showed that snakes feed mainly on small vertebrates: rodents (house mouse, red-tailed gerbil, Turkestan rat), birds, reptiles (swift foot-and-mouth disease), which they eat alive; however, prey kills larger prey with the help of poisonous teeth. Young individuals prefer various lizards and insects. In general, the intensity of feeding of snakes depends on the season of the year and temperature factors. In spring and autumn, they lead a daytime lifestyle, in summer they can eat both during the day and at night.

Conclusions:

1. The gland of the Duvernois snake of the genus *Coluber karelini* is divided into 2 sections: anterior and posterior. According to its morphological structure, it belongs to exocrine, simple branched tubular glands of a mixed type (protein-mucous).

2. The secretory tubes of the posterior section of the gland line the serous cells, and the secretory tubes of the anterior section are mucous.

3. Serous cells of the gland secrete according to the microapocrine type, and the mucous cells of the gland are characterized by merocrine secretion with the release of the secretion through the hole in the cell membrane.

References:

[1] Abubakirova M.E. Features of biology, ecology of a number of snakes, gray monitor lizard, secretion and mechanism of action of their poisons, Tashkent, 1997.

[2] Shakarboev E.B., Akramova F.D., Azimov D.A. Trematodes - parasites of vertebrates in Uzbekistan (structure, functioning and bioecology), Tashkent, 2012.

[3] Kamilova Sh.I. Ecological and biological characteristics of snakes (Colubridae family) and membrane-active properties of their venoms, Tashkent, 2000.

[4] Contrera MGD, Lopes RA, Costa JRV, Petenusci SO, Lima-Verde JS: The histology of salivary glands in the Colubrid snake Sibynomorphus mikanii (Schlegel, 1837). Can J Zool, 61, 936-941, 1983.

[5] Nicolas Vidal., Colubroid systematics: evidence for an early appearance of the venom apparatus followed by extensive evolutionary tinkering / Journal of Toxicology: Toxin Reviews, Volume 21, 2002 - Issue 1-2 // https://doi.org/10.1081/TXR-120004740.

[6] Antoniazzi M, Oliveira L, Zaher H, Jared C: Morphological characterization of the infralabial glands of ‘‘goo-eater’’ snakes (Colubridae: Dipsadinae). In, Abstracts of Fifth World Congress of Herpetology. Stellemboch, South Africa, 119, 2005.

[7] Oliveira L, Jared C, Prudente ALC, Zaher H, Antoniazzi MM: Oral gland in dipsadine “goo-eater” snake: Morphology and histochemistry of the infralabial gland in Atractus reticulatus, Dipsas indica and Sibynomorphus mikanii. Toxicon, 51, 898-913, 2007.

[8] Vidal N: Colubroid systematics: Evidence for an early appearance of the venom apparatus followed by extensive evolutionary tinkering. J. Toxicol. Toxin Rev, 21 (1-2): 21-41, 2002.

[9] Esra Akat et at Histochemical and Histological Investigations on Duvernoy's Gland in Natrix tessellata (Squamata: Colubridae), Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 17(2):285-289, 2013.

[10] Hayes HK, Lavin-Murcio P, Kardong KV: Delivery of Duvernoy's secretion into prey by the brown tree snake, Boiga irregularis (Serpentes: Colubridae). Toxicon, 31, 881-887, 1993.

[11] Kenneth V. Kardong Colubrid snakes and Duvernoy's "venom" glands/ Toxin Reviews 21(1-2):1-19 // DOI: 10.1081/TXR-120004739.

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