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ORIGINAL ARTICLE

COMPARATIVE ULTRASTRUCTURAL STUDY OF THE SALIVARY GLANDS OF TWO HEMATOPHAGOUS LEECHES (ANNELIDA, CLITELLATA, ARHYNCHOBDELLIDA) IN IRAQ

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ABSTRACT

During July - August of 2021, thirty-one leeches were collected from two localities in Erbil and its suburbs for studying the morphological features of jaws, denticles, and salivary gland cells. Leeches were two blood-sucking species; *Hirudo orientalis* (Utevsky & Trontelj, 2005) (Family, Hirudinidae) and *Limnatis paluda* (Tennent 1859) (Family, Praobdellidae). The investigations conducted using a stereomicroscope (SM) and scanning electron microscopy (SEM). *H. orientalis* jaws were white and rigid, bearing sharp teeth, while *L. paluda* jaws were gray and soft bearing fewer blunt teeth with plentiful papilla and both are monostichodont. In the present study, the salivary glands of adult leeches were examined by SEM. They are composed of unicellular glands arranged in grape patterns with spherical, ovoid, and pear shapes in various cell sizes; the cell bunches of gland cells were highly developed and interconnected to one another by tiny channels. A bigger canal that led to the jaws was created by combining channels from each bunch.

Keywords: Annelida, *Hirudo*, Leeches, *Limnatis*, Salivary gland.

INTRODUCTION

Leeches are cylindrical, flattened ectoparasites that feed on blood or are carnivorous members of the phylum Annelida, class Clitellata; despite the numerous terrestrial and marine species, freshwater leeches predominate (Sawyer, 1986). Leeches are segmented, hermaphrodites, blood-feeding worms that can infect humans, pets, wildlife, and invertebrate species; these are distinguished by two distinct suckers, the anterior and posterior, and inside the front sucker is where the leech's mouth is situated (Daniel and Swayer, 1975; Ayhan *et al*., 2021).

Jaws and velum are typically found in the buccal cavity of hematophagous species, which also have large mouths; having either one (monostichodont) or two (distichodont) rows of denticles, three muscular jaws are present. The animal may extract blood and lymph from wounds by cutting through the body surface of hosts with its calcified teeth and continue

sucking blood with the help of the secretion of their salivary glands (Fretter and Graham, 1976; Orevi *et al*., 2000).

Bioactive chemicals found in the saliva of vampire leeches, such as *Hirudo* sp., enable the feeding and preservation of eaten blood as well as the possibility of significant medical advantages to the host. Due to their hematophagous nutrition habits, medicinal leeches have been used for centuries in bloodletting for treating numerous diseases (Hirudotherapy). By transmitting different bioactive compounds to their hosts, leeches have developed the capacity to regulate many mechanisms within their hosts (Joslin *et al*., 2017).

Saliva is produced by single-celled salivary glands in the frontal region of the body of jawed medicinal leeches; these glands are positioned between the muscle fibers that link the jaws to the body wall. From the cell body to the jaw, the individual salivary cell sends a single duct, which terminates in a small aperture between the calcified denticles of the jaw (Wuttke *et al*., 1989).

There are 6 species of medicinal leeches in the genus *Hirudo* (Linnaeus, 1758), involving *Hirudo medicinalis* Linnaeus, 1758 (European medicinal leech), which has been studied in detail but others do not get sufficient importance including *Hirudo oreintalis* (Bahmani *et al*., 2013; Saglam *et al*., 2020).

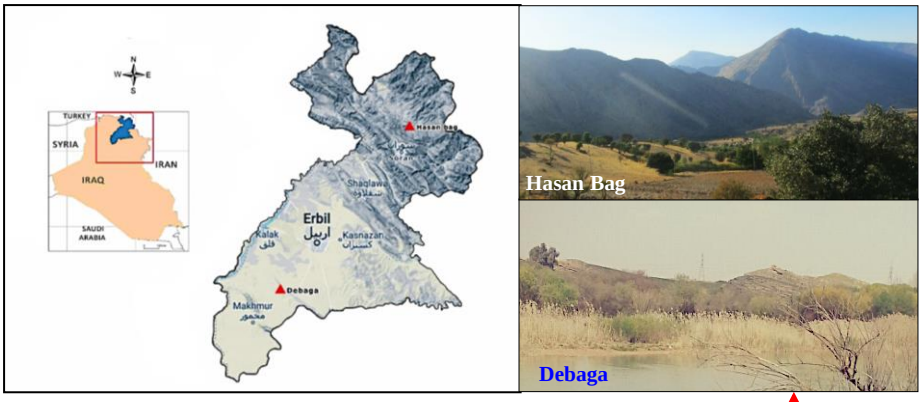
Nasal leeches belong to the taxon identified as *Limnatis* Moquin-Tandon, 1827. Mammals, particularly humans, have been observed to have this genus' leeches invade their nasopharynxes (Almallah, 1968; Boye and Joshi, 1994). Only three species belonging to *Limnatis*: *L. nilotica* (Savigny, 1822), *L. bacescui* Manoleli, 1972, and *L. paluda* (Tennant, 1860) were identified (Sawyer, 1986). In spite of the fact that this species cannot puncture the dermis of humans and animals , it inflames and damages the oral or nasal mucosa by sucking blood (Bahmani *et al*., 2013). They typically adhere to the animal's buccal cavity or pulmonary tracts after ingesting infected water; the species in this genus are crucial for research in parasitology, veterinary science, and medicine (Arfuso *et al*., 2019). Among these three species, *L. nilotica* gets much investigation due to their dominant distributions (Utevsky *et al*., 2022).

The purpose of the current study is to clarify the detailed structure of the two hematophagous leeches' salivary glands *H. oreintalis* and *L. paluda* which differ in their rote of blood feeding, moreover discuss the structure and appearance of their jaws which are related to their feeding mode also.

MATERIALS AND METHODS

Specimens' collection: During July and August of 2021, many water bodies in Erbil Province and its suburbs were searched for collection. Seventeen specimens of *Hirudo orientalis* (Utevsky & Trontelj, 2005) were observed in springs in the Hassan Bag Mountain (N 36° 43’ E 44° 38’) as mentioned by Alishah (2016), While fourteen specimens of *Limnatis paluda* (Tennent, 1859) were gained inside springs, streams, and small lakes in Debaga Township (N

35° 30’ E 43° 45’). Adult active leeches were hand-collected and placed in glass jars, covered with a smooth minute porous cloth to prevent escape. Leeches were kept alive in the laboratory and transferred to a glass aquarium of 30cm (W) x 27cm (H) size, filled with non-chlorinated water. The water was regularly maintained by replacing it every day.



Map (1): Map of Iraq presenting specimens' collection sites (▲).

Specimens' identification: The adult leeches were anesthetized in 10% ethanol for about 5 min. Identification of the worms was done under an Olympus SM depending on their the following external characters; general shape of body, size of mouth, form of suckers, number and arrangement of eyespots (ocelli), papillae, sensillae, copulatory gland pore, the number of annuli per somite (segments), shape and location of male and female gonogpores (Sawyer, 1986; Schenkova´ *et al.*, 2021). For detecting their feeding organs including salivary glands, four specimens of each species were dissected and then cleaned with distilled water (Ayhan *et al*., 2021). Dissection started from anterior to the posterior part along the ventral side on paraffin wax blocks, exposing three rigid jaws and one dorsomedial and two ventrolateral salivary glands. The salivary gland cells have to be as clear as possible and visible, the area around them was cleared from cerebral ganglia, crop, overlying muscle, and connective tissues. Photographs of whole specimens were taken using digital camera adapted to the eye lense.

SEM technique: Anesthetized leeches had their jaws and salivary glands dissected, after which they were fixed overnight at 25° c in 2.5 percentages of glutaraldehyde PH 7.2. After being cleaned 3 times with phosphate buffer solution for 20 minutes, the fixed tissues were subsequently dehydrated using alcohol at increasing concentrations (thirty, fifty, sixty, seventy, eighty, ninety, ninety-five, and hundred) percentages for each concentration in twenty minutes. Step-by-step ، concentrated solutions were applied to the dehydrated tissues (EtOH 1:3, 1:1, 3:1)of acetone for 15 minutes per step, and then transferred to 100 % acetone for 30 minutes. Every specimen was dried in a hood for three days. Ultimately, drying specimens were placed on SEM stubs with both-sided tape, and the specimens were gold coated by DSR1 desk sputter coater in 200 Angstrom for 30 minutes, some samples were silver coated with DSR1 desk sputter coater in 250 angstroms for an hour (Kwak *et al*., 2021).

Comparative ultrastructural study of the salivary

Coated samples were viewed with QUANTUM 450 SEM in the Scientific Research Center, Soran University, Erbil, Iraq.

RESULTS AND DISCUSSION

Kingdom: Animalia
Phylum: Annelida
Class: Clitellata
Order: Arhynchobdellida
Family: Hirudinidae
1. Genus: ***Hirudo*** Linnaeus, 1758
Species: ***Hirudo orientalis*** (Utevsky & Trontelj, 2005)
2. Genus: ***Limnatis*** Moquin-Tandon, 1827
Species: ***Limnatis paluda*** (Tennent, 1859)

1. Jaws

Hirudo orientalis: Jaws are located in a large preoral chamber occupies the anterior sucker, the posterior wall of this chamber is a transverse sheet of tissue known as the vellum, this sucker harbors a small mouth which is a triradiate opening in the center of the vellum (Pl. 1). Two symmetrical ventrolateral and single dorsomedial jaws have been seen, comprising an isosceles, triangle, bright white, smooth and rigid jaws (Pl. 2). The bladelike sharp jaws were seen bearing sharp teeth at the average 80 (range 70-91) per jaw. The jaws are trignathous, monosticodont coated with cuticles except for denticles (Pl. 3). Scant papillae are dispersed on both sides of each jaw (Pl. 3).

Limnatis paluda**:** Anterior sucker occupies a long narrow cut or depression transverse sheet of tissue as three lobes are called median ventral furrow on the ventral surface of the oral sucker which is not observed in *Hirudo* (Pl. 1). Three jaws are situated in the oral cavity; an equilateral triangle is made by a symmetrical couple of ventrolateral jaws and single dorsomedial jaw, and smaller than those of *Hirudo* (Pl. 2). Jaws are gray with black specks that are circular and flexible, they fluctuate toward and away from each other and activated by muscle attached to their bases. Each jaw bearing a line of denticles with bumpy surfaces and flat tops at an average of 43 (range 37- 48) per jaw was seen on their edges (Pl. 3) There were abundant papillae detected on the jaws' double sides. In agreement data with Kovalenko and Utevsky (2015), *Hirudo*'s jaws are stiff, milky white, and glow. It has a line of sharp, pyramid-shaped denticles that are positioned next to one another.

The bite of *Hirudo* is a characteristic triradiate or Y-shape incision made by the three scalpel-like jaws (Sawyer, 1986). Arfuso *et al*. (2019) mentioned that the jaws of *Limnatis nilotica*, closely related to *L. paula*, seemed spherical, spongy, pale grey, and had more distinct knots.

Disparity, *Hirudo* is an ectoparasite due to its pointed teeth and stiff jaws; *it* will puncture its host's dermis to feast on repletion itself when the opportunity presents itself because meals may not always be accessible (Orevi *et al*., 2000).

On the jaw of *H. medecinalis* and *H. orientalis*, the size of the denticles and the quantity they are inversely associated with are observed, therefore the tiny denticles they have, the more teeth are there, while in *H. verbana* was quite different from them, with larger denticles and fewer numbers which allows an efficient piercing of the thick dermis of human and animals. It shows a representation of *Limnatis* jaws with papillae on both sides, a line of tiny denticles on the edge, and a salivary cell ductulus at the termination (Moquin-Tandon, 1827). While Orevi *et al*. (2000) did not detect openings in the teeth of *L. nilotica* but suggested that the papillae might be added to the discharge of salivary secretion of *Hirudo*'s denticle pore; the opposite was found in this study, we detected apertures between the teeth of *L. paluda* (Pl. 3).

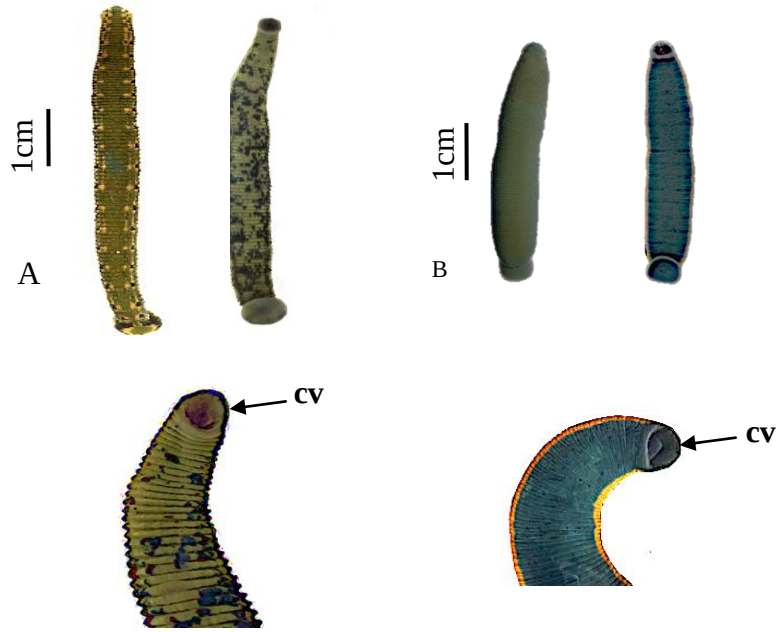


Plate (1): Adult leeches, dorsal and ventral view; (A) *H. orientalis*, (B) *L. paluda*. Buccal cavity (cv).

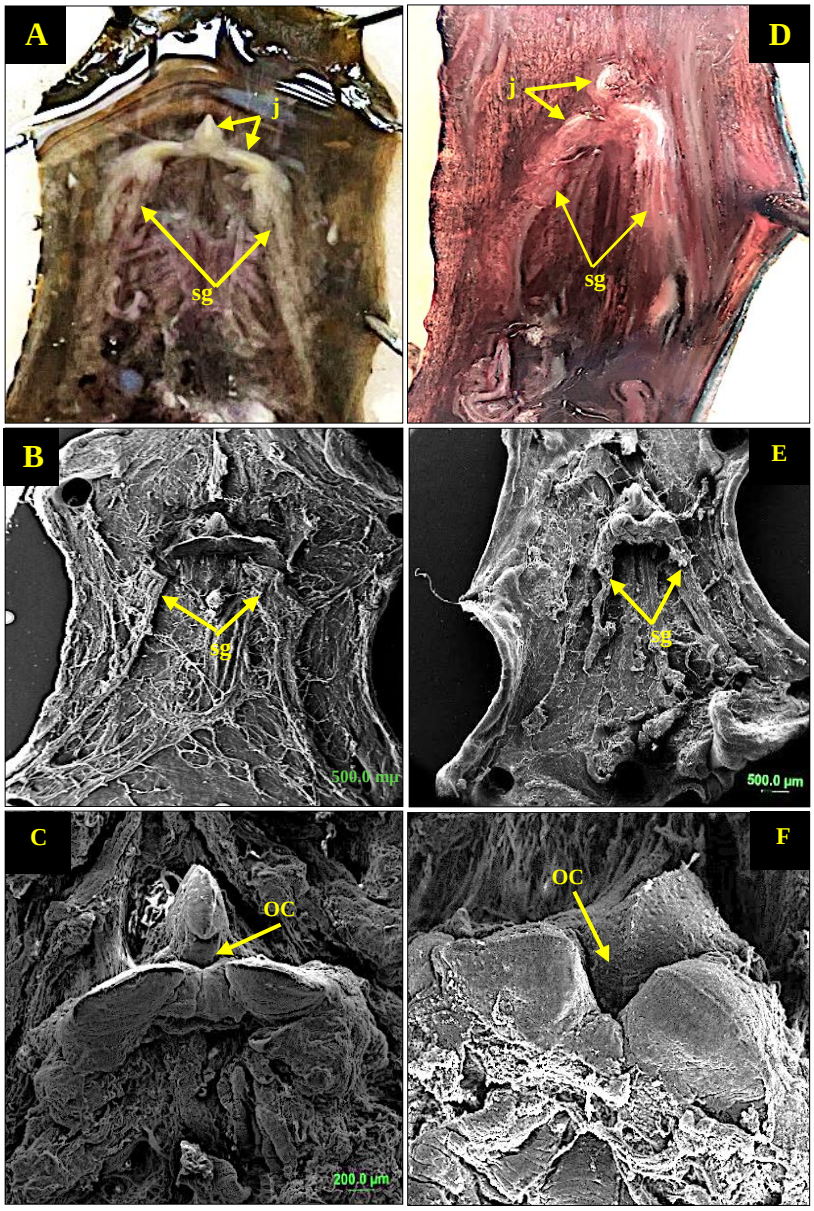


Plate (2): Jaws (j) and salivary glands (sg); (A, B, C) *H. orientalis*, (D, E, F) *L. paluda*. [OC: oral cavity; (B, C, E, F) SEM photographs].

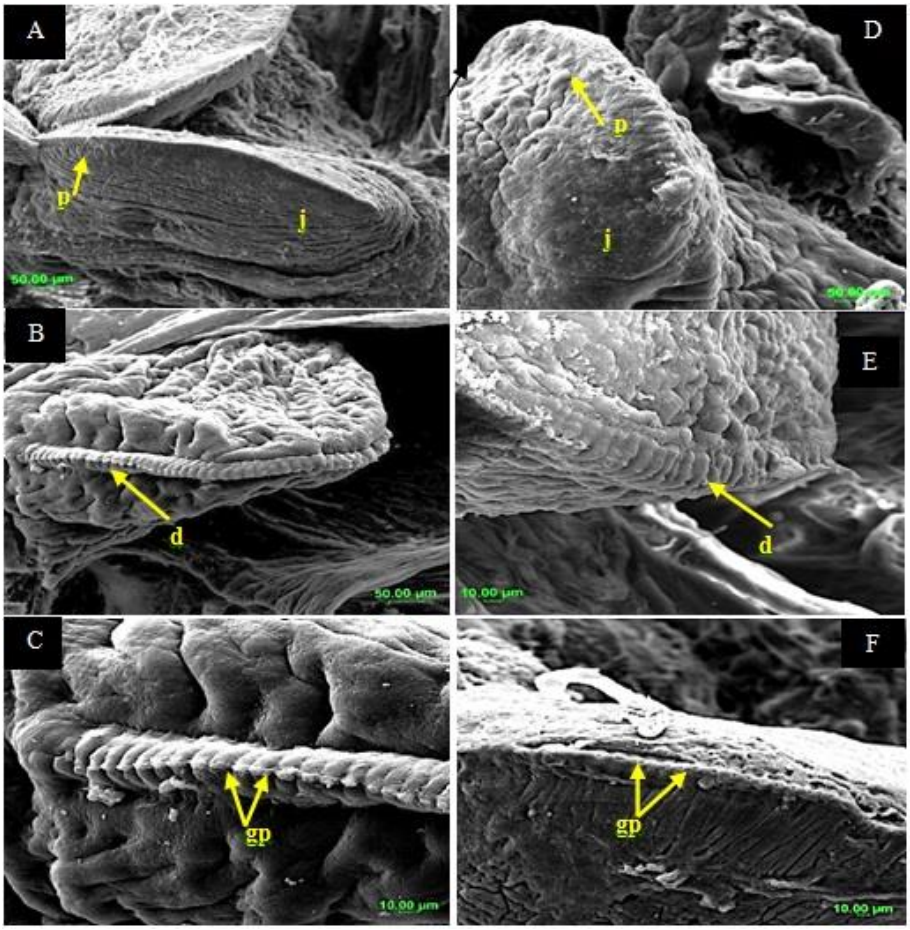


Plate (3): SEM photographs showing; Jaw (J), Denticles (d), Papillae (p) and salivary gland pores (gp) of (A, B, C) *H. orientalis* and (D, E, F) *L. nilotica***.**

2. Salivary glands

***Hirudo orientalis*:** Three separated glands appear as clusters or grapes or strips that are extended from each jaw, spread around the cerebral ganglia, few anterior the crop, located approximately between segments V to XII, they are surrounded by muscles and connective tissues (Pl.4).

***Limnatis paluda*:** General appearance is the same as *H. orientalis* but it is located near position in segments (V - XI) (Pl. 4). Lemke *et al*. (2013) reported that the mature *H. verbana* conical-shaped salivary cells are positioned within segments V to IX, while those of *H. medicinalis* are observed in segments (V - XI) (Marshall and Lent, 1988).

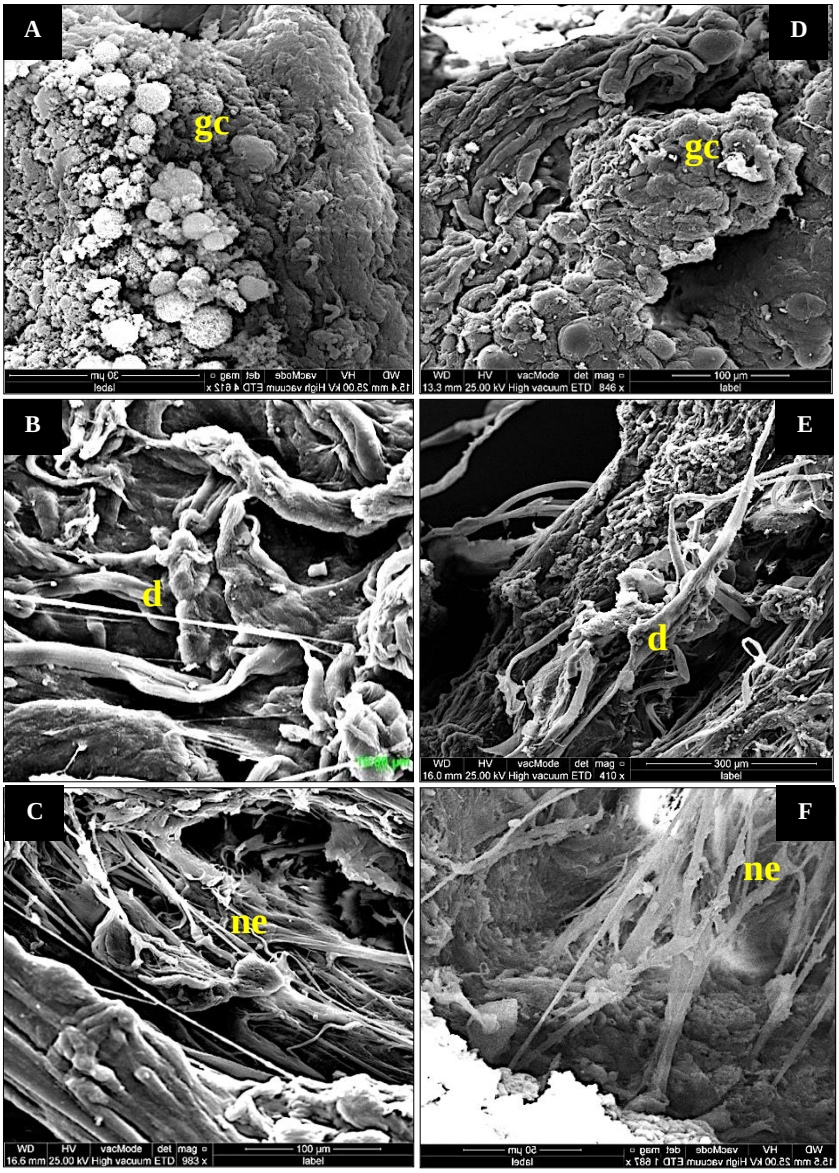


Plate (4): SEM photographs representing; Salivary gland cells(gc), ducts(d) and network (ne), (A, B, C) *H. oreintalis*, (D, E, F) *L. paluda.*

3. Salivary gland cells

***Hirudo orientalis*:** When examined with SEM, thousands of single-celled and 3 of the detected glands were encased in a capsule of connective tissue (Pl.4). The individual gland has indentation cells that were connected to the ducts in a grape-like manner. The cells are arranged in grape-like patterns, spherical, ovoid, and pear-shaped with cell sizes 16 to 78 micrometers after, unconnected to each other. Sulci on the smooth outside surface of the cell were visible, and it appeared as a collection of different-sized cells with system channels interconnecting salivary gland cells to ducts in addition to the thousands of secretory cells. Three different duct configurations were seen in the salivary glands: thin ducts connecting the salivary gland cells and the salivary canal, bigger ducts that appear to travel toward the jaws, and fibrils ducts that appear as tiny capillaries on the outside of gland cells (Pl. 4).

***Limnatis paluda*:** Same appearance and arrangement as of *Hirudo*, but while they had different cell diameters of 13 to 65 micrometers (Pl. 4). Based on the current observational and multiple prior histological investigations (Van der Lande,1968; Mishra and Dev, 1976), it is evident that these jawed leeches' salivary cells are single cells and free cells of different measurements that are assembled in grape-like designs and are matching with other representations of leech salivary glands (Lemke *et al*., 2013; Saglam *et al*., 2020).

Marshall and Lent (1988) and Lemke *et al*. (2013) observed that the somata of *Hirudo medicinalis* are typically circular or elliptical and have a dimension of thirty to two-hundred micrometers. A soma projects a solitary ductule, toward the base of each of the three jaws. However, the *H. verbana* salivary gland cells ranged in size from sixty to a hundred micrometers (Lemke *et al*., 2013); while described as nourished leeches have similar cells, they are significantly tiny and hardly distinguishable. Salivary glands of the enormous amazonian leech, *Haementeria ghilianii*, are ten times bigger than those of *H. verbana* and have a coarser outer layer (Walz *et al*., 1988). In comparison, the salivary gland of *H. verbana* has developed an interconnected network of fibers and saliva ducts (Wuttke *et al*., 1989).

In their study, Saglam *et al*. (2020) mentioned the fact that mature *H. verbana* salivary glands presented as a collection of oval cells of various sizes, with a network of channels tying salivary cells to the ducts and three distinct duct morphologies. The salivary gland cells produce various bioactive compounds that makeup saliva, which is subsequently combined in the previously reported complicated channel system (Marshall and Lent, 1988).

CONCLUSION

The precise morphology of the jaws and salivary glands of *Hirudo orientalis* and *Limnatis paluda* were determined. Both species have in a grapes designed salivary glands that are extended from the jaws and surrounded by muscle fibers between crops and the body wall. Salivary gland cells are unicellular and vary in shape and size, the salivary gland of *Hirudo* in length is longer than *Limnatis* but in general, *Limnatis* cells are smaller than *Hirudo*. The jaws of *Hirudo* are firm, white, larger than *Limnatis* grey and soft and, both species are papillated but *Limnatis* papilla is more than *Hirudo*. *Limnatis paluda* comparatively has a

small number of denticles so due to unable to pierce the skin, while *Hirudo orientalis* have larger and numerous denticles therefore are able to incision.

CONFLICT OF INTEREST STATEMENT

The results of the current study are part of the requirements of MSc thesis in Invertebrate Zoology, Department of Biology/College of Science-Salahaddin University/Erbil-Iraq for the first author. We declare that there is no conflict of interest between the authors. We confirm that all the pictures in the manuscript belong to us. We note, in this study, that there is no conflict of interest regarding the use of the laboratory of College of Science.

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دراسة مقارنة للتركيب الدقيق للغدد اللعابية لإثنين من ديدان العلق الماصة للدم (Annelida, Clitellata, Arhynchobdellida) في العراق

هدى صادق بلال و شيروان طيب احمد
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تأريخ الاستلام: 2022/11/26، تأريخ القبول: 2023/2/26، تأريخ النشر: 2023/6/20

الخلاصة

درست السمات المورفولوجية للفكين والأسنان وخلايا الغدد اللعابية لنوعين من ديدان العلق الماصة للدم: *Hirudo orientalis* (Utevsky & Trontelj, 2005) عائلة Hirudinidae و *Limnatis paluda* (Tennent 1859) عائلة Praobdellidae بأستخدام المجهر المجسم (SM) والمجهر الإلكتروني الماسح (SEM). كانت فكوك *H. orientalis* بيضاء وصلبة ، وتحمل أسنانا حادة ، بينما كانت فكوك *L. paluda* رمادية وناعمة وتحمل عددا أقل من الأسنان الحادة مع حليمة وفيرة وكلاهما أحادي الصف. في الدراسة الحالية، تم فحص الغدد اللعابية للعلق البالغ بواسطة SEM؛ وهي تتألف من غدد وحيدة الخلية مرتبة في أنماط العنب بأشكال كروية وبيضاوية أو كمثرية الشكل و بأحجام مختلفة. ومجموعات الخلايا من خلايا الغدة متطورة للغاية ومترابطة مع بعضها البعض بواسطة قنوات صغيرة. ثم يتم إنشاء قناة أكبر تؤدي إلى الفكين من خلال الجمع بين القنوات من كل مجموعة.