

**تسجيل ليرقة الديدان الشريطية من جنس الفلوريسيس (رتبة: التريبانورينكا) من أسماك
أبو شمس إسماعيلية (البلاميطة) بالبحر الأحمر باستعمال المجهر الإلكتروني الماسم**

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تشتمل هذه الدراسة على تسجيل لأسماك البلاميطة كعائل جديد ليرقات الديدان الشريطية من مجموعة التريبانورينكا من البحر الأحمر بمصر حيث صنفت هذه اليرقات تحت جنس الفلوريسيس. وهذه الدراسة هي واحدة من سلسلة دراسات عن أصابه هذه الأسماك بيرقات التريبانورينكا. حيث سجلت الأصابه من ١٨ نوع من الأسماك شملت كلا من أسماك الناجل و الخرمان. ولقد وجدت رؤوس الديدان الصغيرة متوصلة داخل كيس مكون من ثلاث طبقات حيث تغزو هذه الحويصلات تجويف جسم السمكة و أحشاءها. وتشتمل رأس هذه اليرقات على عضوي تثبيت ورقبي الشكل لحافة كل منهما أنغماد مهذب. كما تحمل أربعة لوا مس مغطاة بخطاطيف مرتبة على نظام سبعة صفوف من الأشواك الرئيسية في كل من نصفى حلزون بالإضافة إلى صف من الأشواك ذات الجناحين. و يغطي سطح جسم اليرقة بأكمله زوائد مخملية ريشية الشكل لها خمسة امتدادات إصبعية عدا حافة عضوي التثبيت التي تغطي بزوائد مخملية لها زائدتين إصبعيتين. ولقد قورن هذا النوع من جنس الفلوريسيس بالنوع الذي سبق وصفه من أسماك الخرمان وأسفرت المقارنة على اختلاف النوع الحالي عما سبقه.

**FURTHER STUDIES ON FRESHWATER TURBELLARIA FROM EGYPT:
MACROSTOMUM ISMAILIENSIS N.SP.**

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Key Words : Turbellaria, Freshwater, *Macrostomum*, Egypt.**ABSTRACT**

This worm belongs to Family Macrostromidae (Beneden, 1870), Order Rhabdocoela (Karling, 1940). Class Turbellaria (Ehrenberg, 1931), Phylum Platyhelminthes (Gegenbauer, 1859). The name of the species was suggested to refer to one of the locations "Ismailia canal" from which this species was collected. The length of the worm ranges from 1.0 to 1.8 mm, while the width is about 0.4 to 0.6 mm. Rhabdites are found in large numbers especially at the anterior end. Two kidney-shaped brownish eyes are situated a short distance in front of the mouth aperture. Adhesive gland cells are located ventrally in the parenchyma at the posterior sixth part of the body, forming a circular adhesive disc. The reproductive system plays a principal role in the identification of these turbellarians, where the present species possesses a curved chitinous stylet that reaches about 180 um in length. The stylet is enclosed inside the tubular penial canal. The distal part of the stylet is slightly curved and enlarged as a result of the equal thickening of its bulb.

The present study is an attempt to contribute to the diversity and taxonomy of the turbellarian worms in the local freshwater ecosystems. Few surveys and taxonomical studies were so far carried out on such freshwater organisms of minor taxa, in various parts of the world, especially those related to class Turbellaria.

MATERIAL AND METHODS

Specimens were obtained from 3 different localities of freshwater tributaries of the River Nile (Fig. 1).

Site (1) on Shebin El-Kanater canal which is situated 25Km north of Cairo city.

Site (2) on Ismailia canal in Moustorod district in Cairo.

Site (3) on the River Nile at El-Gamaa bridge, near Cairo

It is to be mentioned that site (2) was chosen to represent a polluted area, while sites (1) & (3) represented

downstream and upstream waters of the River Nile, respectively.

Since most of the collected specimens are known to be attached to aquatic vegetation, they were obtained by taking off one of each of floating plants namely *Elodea canadensis* and of the submerged rooted plants, *Eichhornia crassipes* at each site. These plants were brought to the laboratory, washed with tap water-current to detach different organisms from them. Collected specimens were put into petri-dishes, where they were identified either by the naked eye or under a research microscope and then they were preserved in 8% formalin. Samples were collected seasonally for a period of one year, starting from December 1991. Turbellarian specimens were best studied alive. They were allowed to swim freely at first, then narcotized by adding few crystals of $MgCl_2$ or $MgSO_4$ and compressed to varying degrees between a glass slide and a vaseline coverslip and examined through a compound microscope. Whole mount preparations of the specimens were stained with Borax carmine and eosin.

Histological preparations of the materials were also made. The worms were killed in Steinmann's fluid and fixed in 70% ethyl alcohol and Zenker's fluid. Materials fixed by the former fixative were stained with haematoxylin and eosin, while the other one was treated with Mallory's triple stain. The specimens were then dehydrated in ascending grades of ethyl alcohol and cleared in cedar wood oil. Specimens were embedded in Paraffin wax and sectioned at 6-8 μm thickness. Serial transverse and longitudinal sections of

the studied specimens were obtained for establishing a whole and complex reconstruction of the various organ-systems of the body. Prepared serial sections of the specimens were stained by haematoxylin & eosin and Mallory's triple stain for detection of connective tissue and muscle fibres. Then, specimens were mounted in canada balsam. A camera lucida was used to draw the outline of the stained specimens, while their photomicrographs were taken by a microscope with an automatic camera.

RESULTS AND DISCUSSION

Location: (Fig.1)

Eighty four specimens were collected from the three stations, from December, 1991 to November, 1992. Of them, 43 specimens were obtained from the second station of Ismailia canal in Moustorod district, 35 specimens were collected from the first station at Shebin El-Kanater canal, 25 kilometers north of Cairo and 6 specimens were collected from the third station at El-Gamaa bridge. They were picked up from around the aquatic vegetation such as *Eichhornia crassipes* and *Elodea canadensis*.

External features:

The body of the worm is oval in shape and pale white in colour. Its length ranges from 1.0 to 1.8 mm., while the width is about 0.4 to 0.6 mm. The anterior end of the body is blunt and the posterior end is broad, while its maximum width lies at the end of its first fourth part. The body is externally surrounded by a coat of equal cilia (Figs. 2 & 3-c). Adenal rhabdites (Figs. 2 & 7-

r) are found in large numbers especially at the dorsal and lateral surfaces of the anterior end.

Adhesive gland cells (Fig. 7-agc) are located ventrally in the parenchymatous tissue at the posterior sixth part of the body forming a circular adhesive disc (Fig. 7-add). Two kidney-shaped brownish eyes (Figs. 1 & 3-e) are situated a short distance in front of the mouth aperture (Figs. 3 & 5-m). The intestine (Figs. 2 & 3-in) is located in a central position.

Epidermal Layer:

The epithelial cells have cylindrical shape with oval nuclei and the dorsal epithelial layer is thinner than the ventral one (Fig. 7-dep & vep). The epidermis varies in height ranging from 9 to 12 μm and its cells are interrupted at intervals by bundles of rhabdites (Fig. 7-r), which are much numerous in dorsal layer than the ventral as in *Macrostomum niloticum* (Beltagi, 1972). The sub-epidermal mucous gland cells (Fig. 7-mgc) are found in great numbers especially at the ventral part of the body. The cilia over the body (Fig. 7-c) are about 6 μm in height. The basal granules of the cilia (Fig. 7-bg) aggregate together along the whole outer surface of the body, forming a thick line as in *Macrostomum thermophilum* (Riedel, 1932).

Musculature:

The sub-epidermal muscle layer consists of an outer circular and an inner longitudinal muscle fibres respectively (Fig. 7-cm & Lm). The parenchymatous muscle fibres have a well-developed muscular formation. Dorso-ventral muscle fibres (Fig. 7-dvmf) are scattered in the parenchymatous tissue (Fig. 7-pt).

Mesenchyma:

The mesenchyma is formed of a syncytial plasmodial parenchyma (Fig. 7-pt). Rhabdite gland cells (Figs 4 & 7-rge) are scattered in the parenchymatous tissue, with their oval nuclei. Sub-epidermal mucous gland cells (Fig. 7-mgc) are found in large numbers, especially at the central region.

Digestive system:

The mouth aperture (Figs. 3 & 5-m) is oval in shape, located medio-ventrally at the end of the first fourth of the body and leads vertically to a flask-shaped simple pharynx (Figs. 3 & 5-ph), which is connected dorso-posteriorly to the anterior part of the intestine (Figs. 3 & 5-in) by an aperture which is surrounded by a strong set of circular muscle fibres, acting as a sphincter muscle (Fig. 5-sm).

The epithelial layer of the pharyngeal tube (Fig. 5-pht) is considered to be an invagination of the ventral epidermal layer. The epithelial cells are cylindrical and possessing a strong and thick ciliary lining (Fig. 5-c), reaching about 6.6 μm in height. The basal granules of the epithelial cilia constitute a striking thick line. However, these cells are interrupted by the outlets of the pharyngeal cyanophilic gland cells (Figs. 3 & 5-phc) embedded in the surrounding parenchymatous tissue. Flask-shaped eosinophilic gland cells (Fig. 5-esg) are pouring their fine rounded granules at the mouth region. Dilator muscle fibres (Fig. 5-dilm) extend radially from the distal part of the pharynx towards the ventral sub-epidermal muscle layer. Furthermore, protractor muscle fibres (Fig. 5-prm) are attached to the proximal part of the

pharynx. The oesophagus is absent and replaced by strong sphincter muscle fibres (Fig. 5-Sm) which are situated in between the pharynx and intestine.

The intestine is sac-shaped and its posterior end is usually placed dorsal to the antrum femininum. The length of the intestine is about 0.50 mm, having a narrow tapering posterior end and slightly wavy outer border. The intestinal epithelium (Figs. 3 & 5-inep) consists of large ciliated cylindrical cells, which represent the phagocytic gland cells (Figs. 3, 4 & 5-phg). The height of each cell reaches about 100 µm and the width of 27 µm with long fine cilia which show no basal granules. Conical gland cells, which represent the enzymatic gland cells are also found (Figs. 3, 4 & 5-eng). The outer border of the intestinal sac is surrounded by a fine outer longitudinal layer and inner thick circular layer of muscle fibers (Fig. 7-Im & cm) as in *Macrostomum coxi* (Young, 1976).

The epithelial cells of the intestine are not in close connection with each other, but are separated by the radial branches of the central intestinal lumen. Some crustaceans namely *Cyclops* and *Daphnia* (Fig. 2-cr) are often found in the intestinal lumen as food materials.

Nervous system:

The brain is crescent-shaped and composed of two ganglionic lobes (Figs. 3 & 5-rlb & llb) connected by a short medio-dorsal nerve commissure (Fig. 3-mdc) as observed by Young (1972) in his study on *Macrostomum johni*. Each nerve ganglion has a maximal diameter of 72 µm consisting of a thick fibrillated plasmatic mass, where oval nuclei are embedded (Fig. 7-n). The brain mass

consists of central matrix of fibrous nervous tissue. Each nerve nucleus has a moderate length of 6.2 µm. A thin membrane surrounds the biganglionic brain as described by Braun (1885). Also the brain is surrounded by a muscular sheath of outer thin longitudinal layer and few inner circular layer of muscle fibres (Fig. 5-Im & cm). The brain gives rise to 4 pairs of nerve stems as follows:

- Right and left anterior nerve stems (Fig. 3-rans & lans) extend anteriorly from the antero-median part of the brain mass, giving rise to several branches of anterior nerves.
- Right and left antero-lateral nerve stems (Fig. 3-raIns & laIns) extend anteriorly from the antero-lateral part of the brain, giving rise to numerous nerves, which supply the antero-lateral part of the body, especially at the sub-epidermal layer.
- Right and left lateral nerve stems (Fig. 3-rlns & llns) extend latero-posteriorly forming two parallel longitudinal nerves, right and left latero-longitudinal nerves (Fig. 3-rlln & llIn). They unite together medio-posteriorly into an enlarged tail nerve ganglion (Fig. 3-tug) as in *Macrostomum viridi* (Luther, 1905) and *Macrostomum niloticum* (Beltagi, 1972).
- Right and left postero-ventral nerve stems (Fig. 3-rvns & Lvns) extend ventrally forming a circumpharyngeal nerve ring (Fig. 3-cpr), which has enlarged nerve ganglion (Fig. 3-ng) behind the pharynx, as mentioned in *Macrostomum niloticum* (Beltagi, 1972) and *Macrostomum deltanensis* (El-Said, 1982).

Sensory organs:

The right and left eyes (Figs. 2 & 3-e) are dark brown in colour, reniform and located dorso-posteriorly to the brain mass. The length of the eye ranges from 0.01 to 0.03 mm, while its width varies from 0.01 to 0.02 mm. Each eye has an eye cup (Fig. 5-ec) of about 24.4 µm in diameter. The eye cup consists of numerous small rounded granules of about 2 µm in diameter each. The lens is half moon-like and has a diameter of 19.8 µm. The eye-chamber is slightly oval and has a length of about 30.8 µm. The eye is completely embedded in the brain mass dorso-posteriorly, and it varies in this respect from *Macrostomum tennesseensis* (Ferguson, 1939-1940). Sensory hairs and spines are absent as in *Macrostomum salemensis* (El-Said, 1982).

Reproductive system:

I. Female genital organs:

The right and left ovaries (Figs. 3 & 5-rov & lov) are partially lobed structures and located laterally. The length of each ovary varies from 0.250 to 0.350 mm.

The right and left oviducts (Figs. 3 & rod & lod) join nearly in the mid-line of the body, forming a short common oviduct (Figs. 3 & 5-cod). The female genital atrium (antrum femininum) is nearly rounded in shape (Figs. 3 & 5-af) and reaches a maximal diameter of about 210 µm. The antrum femininum is lined by a single layer of ciliated cubical epithelial cells (Fig. 3-cepc), of about 4.4 µm thickness, while the height of their cilia nearly equals that of the outer epidermal layer. The female genital aperture (Figs. 3 & 5-fga) is located medio-ventrally, nearly at the beginning of the last 6th part of the body.

The vagina was not detected in the examined specimens, while the antrum femininum appeared to be connected anteriorly with the passage cells (Figs. 3 & 5-pc) or "Verschluss apparat" (Luther, 1947). Few foreign sperms (Fig. 5-fsp) were observed with long tails inside the antrum femininum in front of the passage cells. The antrum femininum is the place where mature ovum is fertilized by foreign sperms to form the zygote (Figs. 3 & 5-zg). It is surrounded by a muscular sheath of outer longitudinal and inner circular muscle fibres.

Two types of oval cement gland cells are found embedded in the parenchymatous tissue around the ventral part of the antrum femininum. The first type of the cement gland cells is situated radially (Fig. 5-cyg) and has a cyanophilic secretion in the form of coarse elongated polygonal granules, each of which measures about 5.2 µm in length. The second type of cement gland cells has eosinophilic secretion (Fig. 5-esg) with narrow rod-shaped granules, each of which has a length of about 7 µm and a width of 1.5 µm. They are accumulated in a thick mass, surrounding the first cyanophilic type of granules as in *Macrostomum niloticum* (Beltagi, 1972).

The cement gland cells secrete some adhesive material, causing the egg shells to be attached to solid objects as in *Macrostomum gigas* redescribed by Hyman, (1943).

II. Male genital organs:

The right and left testes (Fig. 3-rt & lt) are elongated structures and located in the parenchymatous tissue antero-latero-ventrally, behind the brain mass as in *Macrostomum georgeense* (Young, 1976).

Each testis is connected with a long, narrow, thin walled vas deferens. The right and left vasa deferentia (Fig. 3-rvd & lvd) extend posteriorly and ventrally to the ovaries, and unite together to open directly into a thin walled oval false vesicula seminalis (Figs. 3, 5&7-fs), of about 160 µm in length and 95 µm in width, surrounded by a very thin muscular layer mainly of outer longitudinal and inner circular fibres. It leads posteriorly to an oval vesicula seminalis (Figs. 3, 5, 6 & 7vs).

The vesicula seminalis reaches about 91 µm in length and about 45 µm in width. The epithelial layer of the vesicula seminalis appeared as syncytial structure with fine fibrillated plasma, in which oval nuclei are embedded. The wall of the vesicula seminalis is covered by an outer longitudinal, inner circular layer and a few diagonal muscle fibres.

The vesicula seminalis leads ventrally to a pear-shaped vesicula granulorum (Figs. 3, 6& 7-vg) through a very short ductus intervesicularis (Figs. 3, 5 & 6-di). The ventral part of the vesicula granulorum is divided into 5 interciliary chambers (Figs. 5, 6-icc) by 5 groups of long cilia. The accessory genital gland cells (Figs. 3, 5, 6&7-ac) are flask-shaped and embedded in the parenchymatous tissue, surrounding the vesicula granulorum.

The distal end of the vesicula granulorum is connected with the proximal part of the curved chitinous stylet (Figs. 2, 3, 5, 7 & 8-st) whose length reaches about 180 µm. The distal part of the stylet is enlarged as a result of the equal thickening of its bulb (Figs. 3 & 5, -dest). The stylet is enclosed inside the tubular penial canal (Figs. 5&7-

plc), which leads to the male genital aperture (Figs. 3&7-nga) lying medio-ventrally far behind the female genital aperture. The penial canal is limited by outer longitudinal and inner circular muscle fibers.

Differential diagnosis:

In identifying this species, the general structure both externally and internally especially the penis stylet was very important. In this respect, it differs from *Macrostomum aegyptium* (Beltagi, 1972) as it has slightly curved proximal part of the penis stylet while the latter species has a proximal bent part nearly forming a rectangle with the main penis stylet axis. Also it differs from *Macrostomum bulbostylum* (Ferguson, 1939-1940) due to the presence of an adhesive disc and the distal part of the stylet is sharply bent.

Moreover, this type of penis stylet differs from those described in *Macrostomum tuba* (Graff, 1882), *Macrostomum pseudoobtusum* (Beklemischev, 1927) and *Macrostomum subterraneum* (Rixen, 1961) regarding its proximal and distal ends of the stylet. Besides, it differs from that described in *Macrostomum riedli*, and *Macrostomum goharii* (Beltagi et al., 2000). Thus, this worm could be considered as a new species, *Macrostomum ismailiensis* n. sp. Its nomination refers to the name of "Ismailia canal" from which this species was collected. It is registered under the code number (TRM1), at the Department of Zoology, Faculty of Science, Ain Shams University, Cairo, Egypt.

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LIST OF ABBREVIATIONS

ac	accessory gland cell	lt	left testis
acg	accessory gland	lvd	left vas deferens
add	adhesive disc	lvn	left ventral nerve
af	antrum femininum	lvnc	left ventral nerve cord
agc	adhesive gland cell	lvns	left ventral nerve stem
anf	annular muscle fibre	m	Mouth
argc	adrenal rhabdite gland cell	mdc	medio-dorsal commissure
c	cilia	me	membrane
cepc	cubical epithelial cell	mga	male genital aperture
cm	circular muscle fibre	mge	mucous gland cell
cod	common oviduct	mov	mature ovum
cpr	circumpharyngeal nerve ring	n	nucleus
cyg	cyanophilic granule	ng	nerve ganglion
cygc	cyanophilic gland cell	pc	passage cell
dep	dorsal epithelium	ph	pharynx
dest	distal end of the stylet	phc	pharyngeal cyanophilic gland cell
di	ductus mtervesicularis	phg	phagocytic gland cell
dilim	dilator muscle	prm	protractor muscle fibre
dimf	diagonal muscle fibre	plc	penial canal
dvmf	dorso-ventral muscle fibre	prm	protractor muscle fibre
e	eye	pt	parenchymatous tissue
ec	eye cup	r	rhabdite
eng	enzymatic gland cell	raInS	right antero latero-dorsal nerve stem
ep	epithelium	rans	right anterior nerve stem
ept	epithelial tube	rem	retractor muscle
epw	epithelial wall	rgc	rhabdite gland cell
esg	eosinophilic granule	rh	rhammite
esgc	eosinophilic gland cell	rlb	right lobe
fe	fertilized egg	rlIn	right latero-ongitudinal nerve
fga	female genital aperture	rlnc	right lateral nerve cord
fs	false vesicula seminalis	rlns	right lateral nerve stem
icm	inner circular muscle fibre	rlvn	right latero-ventral nerve
ilm	inner longitudinal muscle fibre	rod	right oviduct
in	intestine	rov	right ovary
inep	intestinal epithelium	rt	right testis
inl	intestinal lumen	rvd	right vas deferens
lalns	left antero-lateral nerve stem	rvns	right ventral nerve stem
lans	left anterior nerve stem	sm	sphincter muscle
llb	left lobe	st	stylet
llin	left latero-longitudinal nerve	te	testis
llns	left lateral nerve stem	tug	tail nerve ganglion
llvn	left latero-ventral nerve	vep	ventral epithelium
lm	longitudinal muscle	vg	vesicula granulorum
lod	left oviduct	vs	vesicula seminalis
lnf.	longitudinal muscle fibre	zg	Zygote

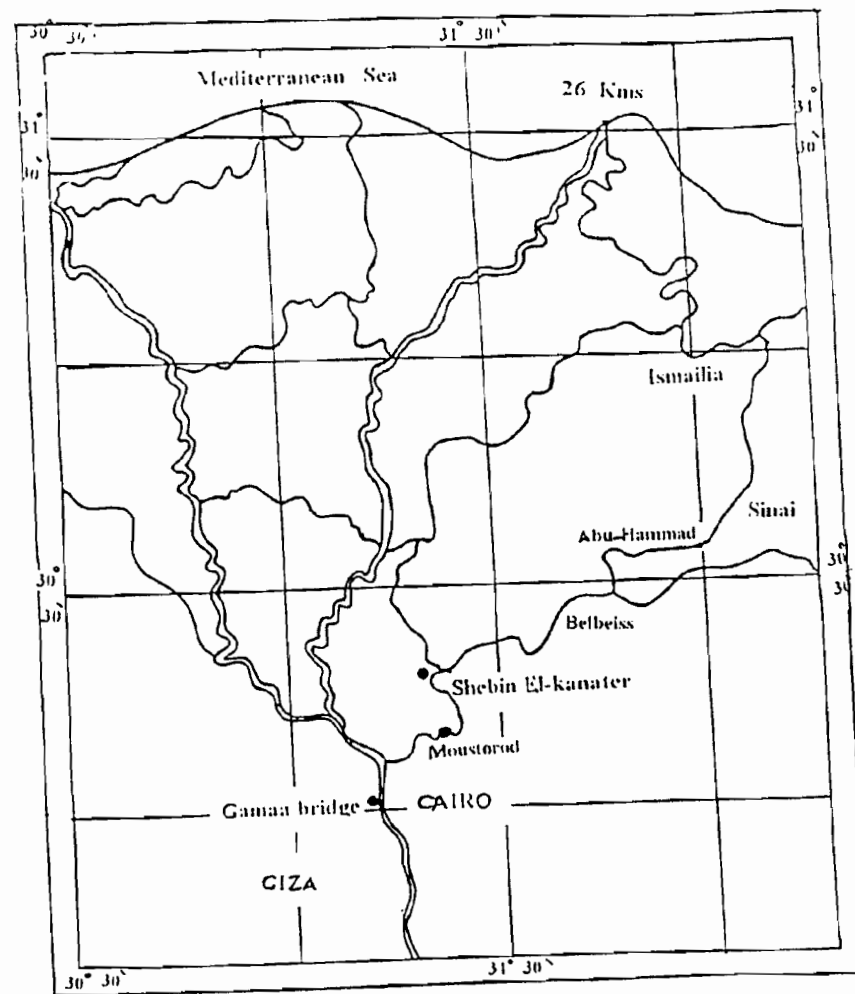


Fig. (1): A sketch map showing sampling stations of the experimental area along the lower Nile.

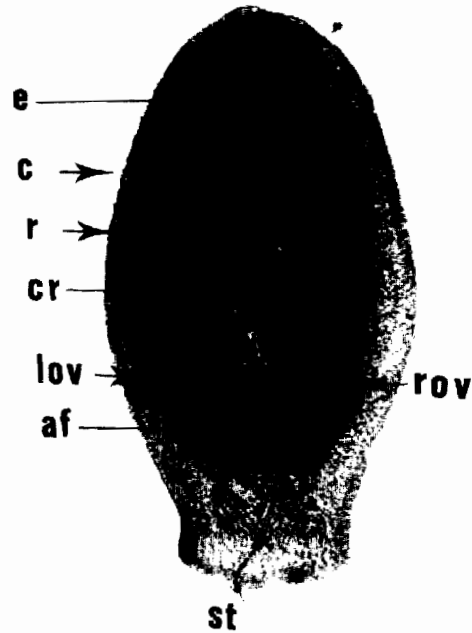


Fig. (2): *Macrostomum ismailiensis* n. sp.

Photomicrograph of a stained specimen. (dorsal view) of external features. Eye (e), cilia (c), rhabdite (r), crustacean (cr), right & left ovaries (rov & lov), antrum femininum (af) and stylet (st).

(X 40)

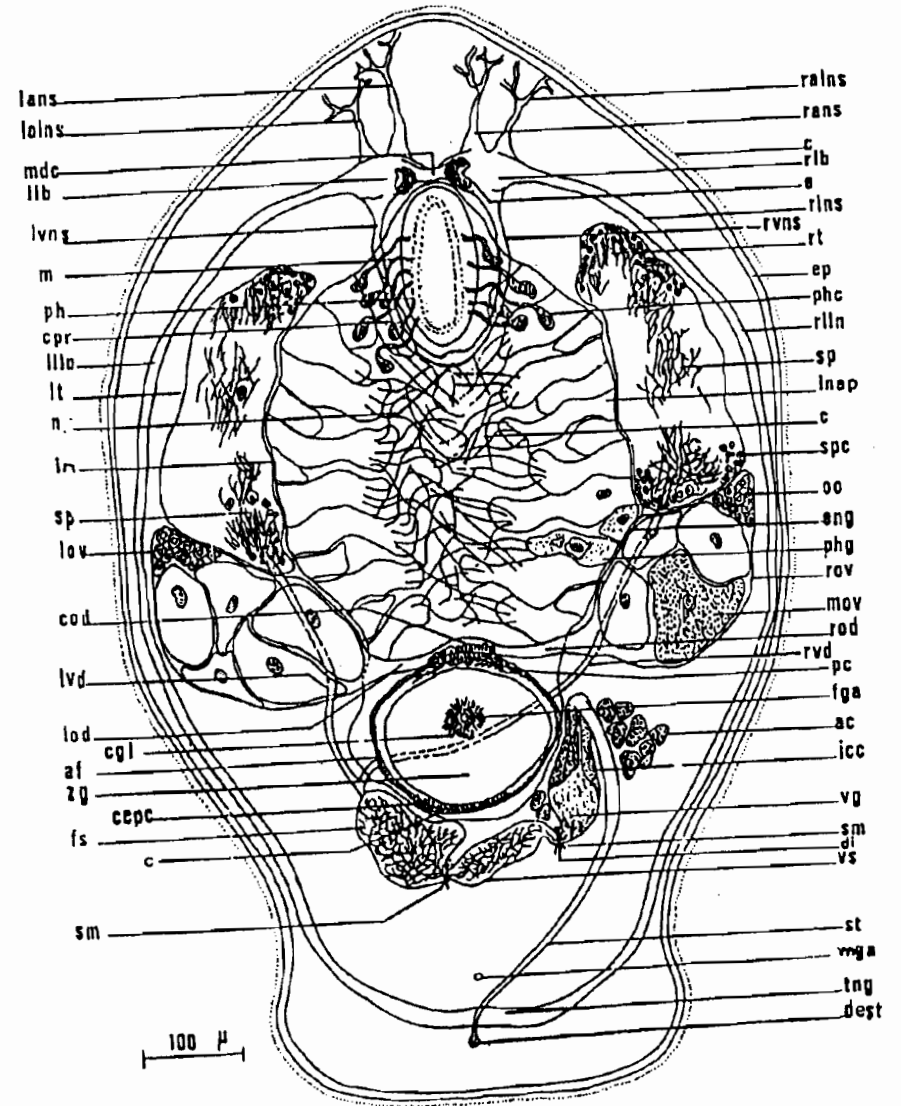


Fig. (3): *Macrostomum ismailiensis* n. sp.
 A diagrammatic representation of the dorsal view showing some of the internal organs.



Fig. (6): *Macrostomum ismailiensis* n. sp.

Photomicrograph of squeezed preparation, showing a large part of the male genital apparatus. Accessory gland cell (ac), vesicula granulorum (vg), interciliary chamber (ic), ductus intervesicularis (di), vesicula seminalis (vs) and stylet

(X 200)

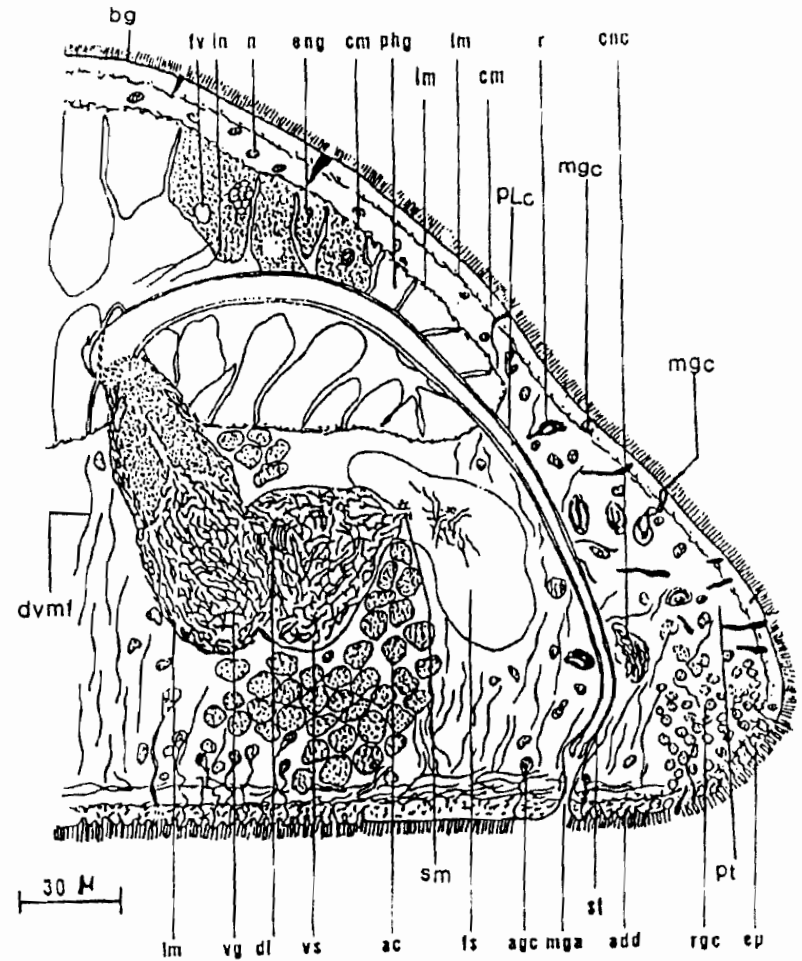


Fig. (7): *Macrostomum ismailiensis* n. sp.

A diagrammatic representation of a lateral part of longitudinal section showing some organs of the male apparatus.



Fig. (8): *Macrostomum ismailiensis* n. sp.
 Photomicrograph of squeezed preparation, showing the
 distal end of stylet (dest). Stylet (St) & Penial canal (Plc).
 (X 640)

ISSN-1110-5350

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العلمية لعلم الحيات

جامعة جنوب الوادي - أسوان

٢٤-٢٨ فبراير ٢٠٠١

العدد الخامس والثلاثون - (D)

دراسات على تيربلاريا المياه العذبة في مصر.

"ماكروستوم اسماعيلينسيس (نوع جديد)"

سمير بلتاجي، عبد الله ابراهيم، أميمة مصطفى

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يتبع هذا النوع عائلة الماكروستوميدي ورتبة وحيدة الجوف وطائفة التيربلاريا وشعبة المقاطحات، ويشير أسم النوع الى أحد المواقع التي تم جمعه منها (ترعة الأسماعيلية)، ويتراوح طول الجسم من ١ الى ١,٨ مم بينما العرض من ٠,٤ الى ٠,٦ مم، وقد لوحظ وجود الزهايتيدات بكثرة على الطرف الأمامي منها، وعلى مسافة قصيرة من فتحة الفم يقع زوج من العيون البنية ذات شكل كلوي، كما تتواجد خلايا غدية لاصقة على الجانب البطنى من النسيج البرانشيمي، مكونة ما يعرف بالقرص الاصق. ويلعب الجهاز التناسلى دورا رئيسيا فى التعرف على هذا النوع، حيث يوجد قضيب كيتينى سحى داخل قناة قضيبية أنبوبية، ويصل طوله حوالى ١٨٠ ميكرون، أما طرفه الخالص فيتصحم نتيجة لتعظ المتأخر على جانبيه.