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# LIGHT AND SCANNING ELECTRON MICROSCOPY OF THREE PARASITIC HELMINTHS FROM FRESHWATER FISHES IN ASSIUT, EGYPT.

(With 5 Plates)

By

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## استخدام الميكروسكوب الضوئى والمجهر الالكترونى الماسح فى وصف ثلاثة من الديدان المتطفلة على أسماك المياه العذبة فى محافظة أسيوط

*نوال عبد السميع محمد مازن ، حسناء محمد ثابت*

تم اعادة وصف ثلاث أنواع من الديدان الطفيلية وهى: ١- بروكمالاناس ليفيكونكس (ويدل، ١٨٦٢ وبيلاس، ١٩٢٣) ، تم دراسة هذا النوع لأول مرة بأستخدام المجهر الالكترونى الماسح فى أسيوط. حيث أظهرت الدراسة وجود ستة شفاه أمامية وأربعة حلمات تحت وسطية فى الجزء الأمامى، وأسنان رفيعة تشبه أسنان المنشار فى قاع تجويف الفم، وثمانية حلمات مستديرة فى الجزء الخلفى. وهذه الصفات قد تكون ذات أهمية كبيرة فى التمييز بين الانواع المختلفة لجنس البروكماليناس. ٢- تم اعادة وصف نوعين من ديدان الرأس شوكيات ودراستها بالمجهر الالكترونى الماسح لتوضيح السطح الخارجى والاشواك الموجودة به لكلا الطفيليين وهما: أ- ريديـنوريكس نيلوتـيكس (محمديـن ١٩٨٩). ب- أكانثوجـيرس (أكانثوسـينتس) تيلابـيا (بـيلاس، ١٩٤٧)، وأظهرت الدراسة تفاصيل هامة وخاصة فى الأشـواك التى تحيط بالبوز والجسم، والتى لها أهمية خاصة فى التمييز بين أجناس وأنواع الرأس شوكيات.

## SUMMARY

During the present study, a nematode and two acanthocephalan parasites of freshwater fishes were collected from Assuit city to be examined by scanning electron microscope (SEM). The surface fine structure of the three parasites, was revealed in order to help their identification and distinction from other allied species. These worms are: 1-*Procamallanus laeviconchus* (Wedi, 1862), Baylis, 1923, in which SEM illustrated: six anterior elevations or lips, four submedian cephalic papillae, minute saw-like teeth at the bottom of the buccal capsule and eight blunt caudal

papillae. These may be important features in differentiation between different *Procamallnus* spp. 2-A- *Rhadinorhynchus niloticus* Mohamadin, 1989. B- *Acanthogyrus (Acanthosentis) tilapiae*, Baylis, 1947. SEM of *acanthocephala* illustrated more detail structures in the proboscis and body spines which are of great importance in generic and species identifications.

***Key words:*** *SEM, Procamallanus, Rhadinochynchus, Acanthogyrus, fresh water fish, Assiut, Egypt.*

## INTRODUCTION

Scanning electron microscope (SEM) makes possible the study of the surface structure which is taxonomically highly significant in the different species of parasites. During investigations of the parasite fauna of freshwater fishes carried out in Assuit Governorate, one nematode and two acanthocephalan parasites were collected from the small intestine and stomach of *Synodontis schall, Lates niloticus, and Oreochromis niloticus.* The nematode *Procamallanus (Spirocamallanus) neocaballeroi* (Caballero-Deloya, 1977) is a specific intestinal parasite of freshwater characid, *Astyanax fasciatus* (Cuvier) in Mexico. It was originally described from Lake Catemaco in Veracruz (Caballero-Deloya, 1977) and recently has been recorded from Cenotes (Sinkholes) in the coastal region of the State of Quintana Roo of Yucatan Peninsula (Moravec *et al.,* 1995 a and b). Information on the development of these nematodes and their larval morphogenesis still remains insufficient (Anderson, 1992). The same concerns congeneric species of the subgenus *Procamallanus,* where the development of only three African and Asian species has so far been studied (Moravec, 1975; Wang and Ling, 1975; Den and Majumder, 1986 and Sinha, 1988). Moravec *et al.* (2000) described 3 new species of *Procamallanus (spirocamallanus)* from the intestine of freshwater fishes in Mexico. Moravec and Van As (2004) recorded five nematode species from stomach and rectum of Spotted Squeaker *Synodontis nigromaculatus.* Moravec (1974 & 1975) described *Procamallanus laeviconchus* from *Clarias gariepinus. P. laeviconchus* has been reported from Egypt, Ghana, Nigeria, Senegal, South Africa, Sudan, Uganda and Zaire (Vassiliades, 1975; Shotter, 1980 and Khalil & Polling, 1997). In south Africa, it has been recorded from *Clarias gariepinus* in dams of the Limpopo River drainage system (Mashego & Saayman, 1981; Boomker, 1982, 1994).

*Acanthogyrus* Thapr, 1927 (*Acanthosentis* Verma & Datta, 1929), belonged to family Quadrigyridae Van Cleave, 1920. Golvan (1959) synonymised *Acanthosentis* with *Acanthogyrus* and reduced *Acanthosentis* to subgenus, based on the number of hooks on the proboscis. The normal subgenus *Acanthosentis* has three circles of eight proboscis hooks. Amin, (2005) described two new species of Acanthocephala, in Japan, *Acanthogyrus* (*Acanthosentis alternat* n.sp & A.(A.) *paraceptaclis* n.sp from Lake Biwa drainage fishes.

The genus *Rhadinorhynchus* Luhe, 1911, a synonym with genus *Nipporhynchus* Chandler, 1934 was related to family Rhadinorhynchidae Travassos, 1923. The characters of *Rhadinorhynchus niloticus* were encountered and decribed by Mohamadin (1989) for the first time in Egypt from the intestine of *Latus niloticus* & *Bagrus bayad* from the River Nile at Quena Province. The present worm was described from the stomach (new habitat) and intestine of *Synodontis schall* & *Oreochromis niloticus* from the River Nile at Assiut province. The presence or absence of trunk spines and the number of cement glands define the family Echinorhynchida (Amin, 1985 and Golvan, 1969).

## MATERIALS and METHODS

The adults of the nematode *Procamallanus laeviconchus* and the Acanthocephalans *Rhadinorhynchus niloticus, Acanthogyrus (Acanthosentis) tilapiae* were collected from the stomach and intestine of *Synodontis schall, Latus niloticus, Bagrus bayad, Bagrus docmac and Oreochromis niloticus* fishes that were purchased from Assiut fishmarket. The three species of worms were washed thoroughly in normal saline and fixed in AFA solution (2% acetic acid, 3% formaldehyde and 70% alcohol). Part of the samples was used for the worm identification by light microscopy.

For SEM studies, samples of the collected worms were fixed in 2.5% glutraldehyde solution in 0.1 phosphate buffer (PH 7.4) for 30 minutes. After washing in the same buffer solution, they were fixed in 1% osmium tetroxide for one hour. Eggs were collected from the uterus of female worms and prepared for SEM (Appleton and Belinda, 1989) and coated with gold palladium, using apolarone 500 sputter coating unit and viewed on Hitachi 5.570 Scanning Electron Microscope at SEM unit in Assiut University.

# RESULTS

**1-a) Light microscopic description of *Procamallanus laeviconchus:***

The body is elongated, filiform and covered with smooth and thick cuticle with distinct transverse striations. The mouth aperture is provided with a chitinous buccal capsule. This is followed by a cylindrical oesophagus which is differentiated into a muscular anterior part of 0.29-0.43 mm length, (average 0.35mm) and a longer glandular posterior one which measures 0.43-0.70mm, (average 0.52mm) (Plate I, Fig.1). The muscular oesophagus is surrounded by a nerve ring at distance 0.15mm from the anterior end in the male and at 0.227mm in the female. The glandular oesophagus opens into the intestine which leads to the anus.

**Male:**

The males are usually smaller and more slender than the females and also fewer in number. The body measures 2.17-3.47mm (average 2.99mm) in length by 0.086-0.13mm (average 0.11mm) in width. The posterior end of the male is sharply curled ventrally and bears 8 pairs of pre-cloacal papillae and 4 pairs of post-cloacal papillae. There are two unequal spicules, the longer one measures 0.06-0.16mm (average 0.09mm) in length and the smaller one is hardly visible (Plate I, Fig 2).

**Female:**

The female is usually longer than the male. Its body measures 3.47-5.86mm (average 4.7mm) in length by 0.13-0.21mm (average 0.18mm) in width The posterior extremity of the body is conical in shape and provided with blunt processes or papillae. The anus is situated near the posterior end at distance 0.06-0.10mm (average 0.077mm). Vulva is situated at the middle of the body (Plate I, Fig 3). The egg is very small in size and measures 25.2-43.4 μ(32.4 μ) in length by 14.4-32.4 μ (average 24.17 μ) in width.

**1-b) SEM description of *Procamallanus laeviconchus:***

The electron micrographs showed that the worm has rounded mouth aperature (Plate II; Fig.1) surrounded by six elevations or lips and four submedian cephalic papillae (Plate II; Fig. 2). There are minute, saw – like teeth at the bottom of the buccal capsule (Plate II; Fig. 3). Deirids are very small, simple and situated behind the anterior end (Plate I I; Fig. 4).

The posterior extremity of the male body is conical in shape and provided with eight blunt processes or papillae (Plate II; Fig. 5). The anus is situated near the posterior end (Plate II; Fig. 6). The posterior

extremity of the male is sharply curled ventrally and bears caudal papillae (Plate II; Fig.7). SEM revealed that The body is covered with smooth and thick cuticle with distinct transverse striations (Plate II; Fig. 8).

**2-a) Light microscopic descrioption of *Rhadinorhynchus niloticus:***

**Male:**

The body is long, slender and measures 7.5-10.6mm (average 9mm) in length with a maximum width of 0.47-1mm (average 0.8mm). It is covered anteriorly by irregularly arranged spines which cover about 1.5-3.1mm (average 2.6mm) of the ventral surface but only 1.9-2.5mm (average 2.2mm) of the dorsal surface (Plate I; Fig. 4). Proboscis is 0.86-303mm (average 1.77mm) long by 0.26-0.56mm (average 0.39mm) wide, armed with 16-22 longitudinal rows of hooks, each of them cosists of 22-26 strong recurved hooks (Plate I; Fig. 6).

**Female:**

The female body (Plate I, Fig. 5) measures 9-12mm (average 10mm) in length and 0.56-1mm (average 0.79mm) in maximum width. Proboscis measures 0.47-4.6mm (1.46mm) in length by 0.2-0.3mm (average 0.29mm) in width. It bears 16-22 longitudinal rows of hooks of 22-26 hooks each. Each hook is strongly recurved and composed of two parts, blade and root. Eggs are elongated with polar prolongations. Each measures 0.12-0.16mm (average 0.14) in length by 0.014-0.018mm (average 0.017mm) in width (Plate I; Fig. 7).

**2-b) SEM description of *Rhadinorhynchus niloticus:***

SEM micrographs confirm the light microscopic desciption. The body consists of an anterior proboscis, a neck and a trunk. The proboscis is cylindrical, long and provided with numerous rows of strongly recurved hooks (Plate III; Figs 1,2,3). The anterior half of the trunk is armed with irregularly arranged short triangular spines (Plate III; Fig 4). These spines are more extensive ventrally than dorsally. There are wrinkles or irregular striations at the trunk region which disappear near the last third of the body (Plate III; Fig. 5). The posterior end of the worm contains a genital pore which is terminal in position (Plate III; Fig. 6).

**3-a) Light microscopic description of *Acanthogyrus (Acanthosentis) tilapiae:***

The body is elongated, cylindrical, gradually enlarging from anterior end to its middle and narrowing from there to posterior extremity. The body is surrounded by spinose cuticle at the trunk region. Proboscis is globular, short, with three transverse rows of six hooks

each. There is a pair of lemnisci projection posteriorly. They are slender, cylindrical, subequal and much longer than proboscis receptacle.

**Male:**

The body measures 2.21-3.56mm (average 3.03mm) in length by 0.78-1.17mm (average 0.97mm) in maximum width. Proboscis is 0.079-0.097mm long by 0.064-0.083mm wide, with an average (0.088mm X 0.074mm). Proboscis hooks of anterior row are 0.039-0.047mm long (average 0.043mm). The middle hooks of the anterior row are needle-shaped, while the peripheral ones are curved. Hooks of the second row are 0.022-0.034mm long (average 0.028mm). The hooks of basal row are 0.011-0.015mm long (average 0.013mm). There are two unequal lemnisci situated at the first body third; the shorter lemniscus is 0.59-0.80mm (0.72mm) long and longer one is 0.60-0.89mm(0.75mm) long. They are equal in width measuring 0.036mm. The testes are ovoid in shape, tandem, slightly touching each other, subequal and occupying about the middle third of the body (Plate IV; Fig.1).

**Female:**

The body is elongated,with maximum width in the middle region (Plate IV, Fig. 2) . It measures 3.13-6mm (average 5.82mm) in length by 0.87-1.5mm (average 1.22mm) in width. The proboscis measures 0.079-0.126mm (average 0.098mm) in length by 0.072-0.079mm (average 0.076mm) in width. Proboscis hooks are similar to those of the male, with the same arrangement and number. The two lemnisci are easily observed. Eggs are small in size, elliptical or fusiform and measuring 12.6-25.2μ (average 18.72μ) in length by 5.4-10.8μ (average 7.56μ) in width (Plate IV; Fig. 3).

**3-b) SEM description of *Acanthogyrus (Acanthosentis) tilapiae:***

The worm body is elongated and cylindrical (Plate IV; Fig. 4). Proboscis is globular, with three transverse rows of six hooks each (Plate V; Fig. 2). There are wrinkles at the trunk region (Plate V; Fig. 3). The cuticle is provided with transverse rings of cuticular spines (Plate V; Fig. 4). Beneath the cuticle, there is a syncytial hypodermis which contains a number of nuclei, they appear as amoeboid structures on the body surface (Plate V; Fig. 5). The posterior end of the body contains the genital pore (Plate V; Fig. 6).

# Plate I

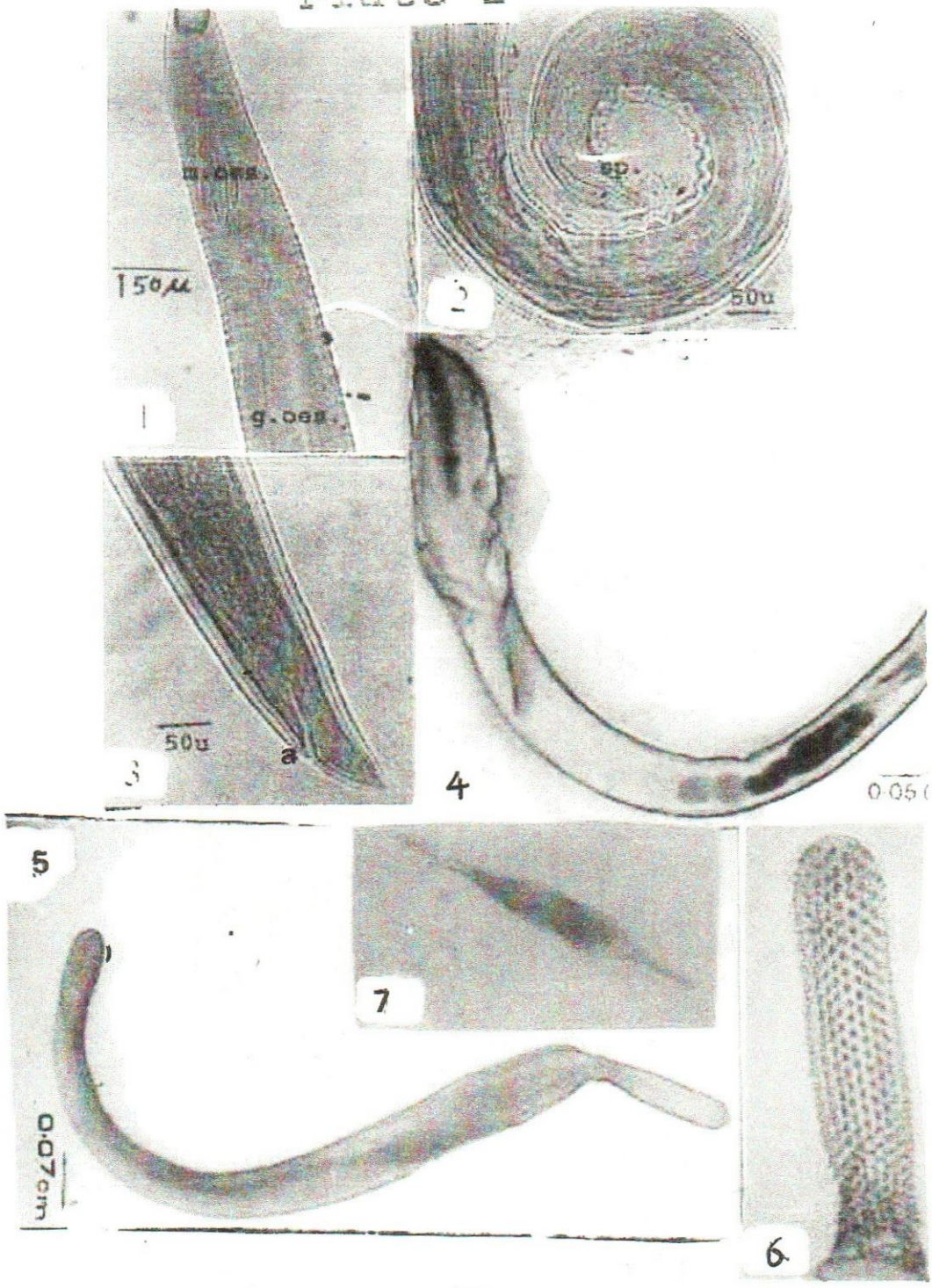
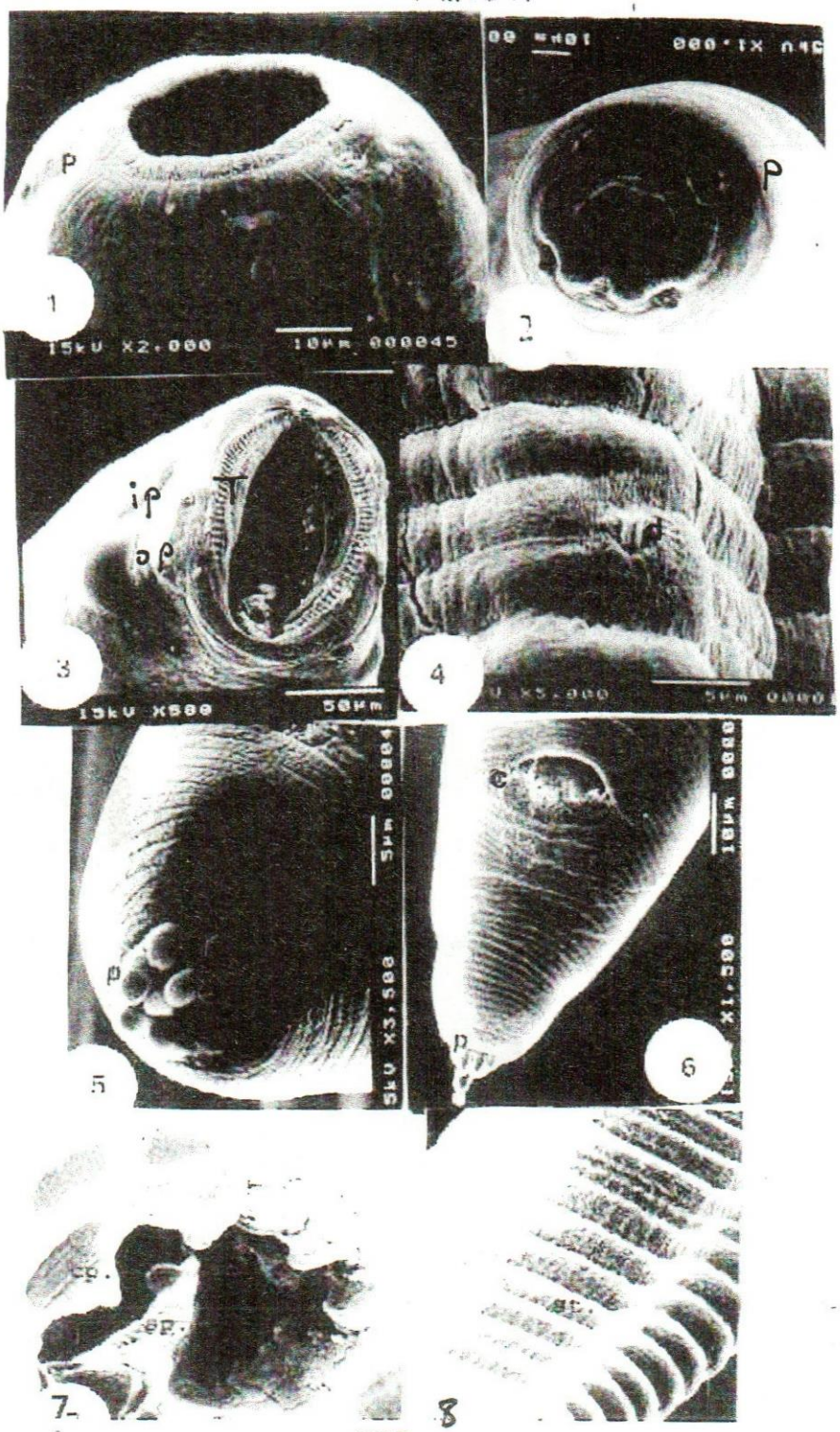


Plate II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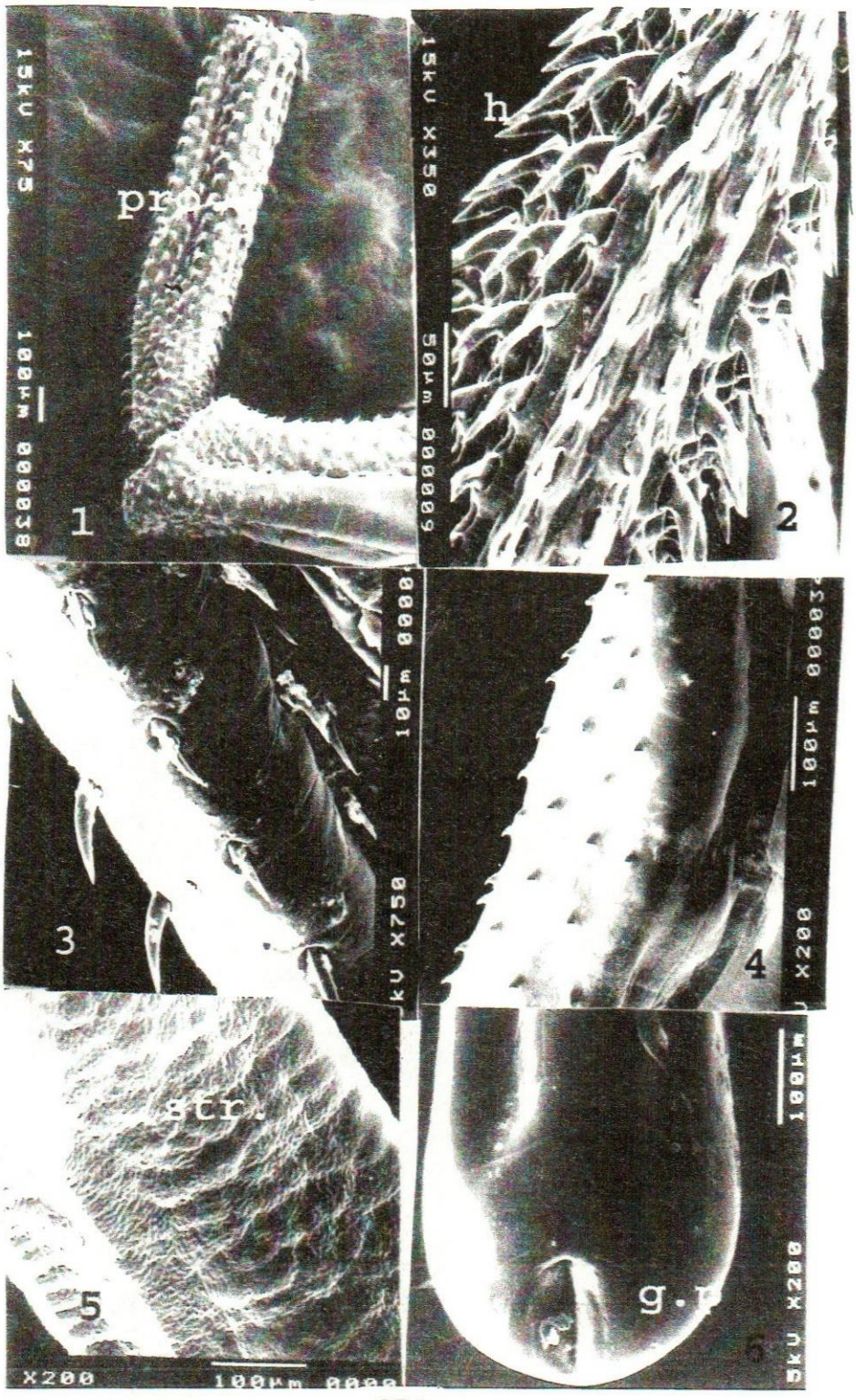
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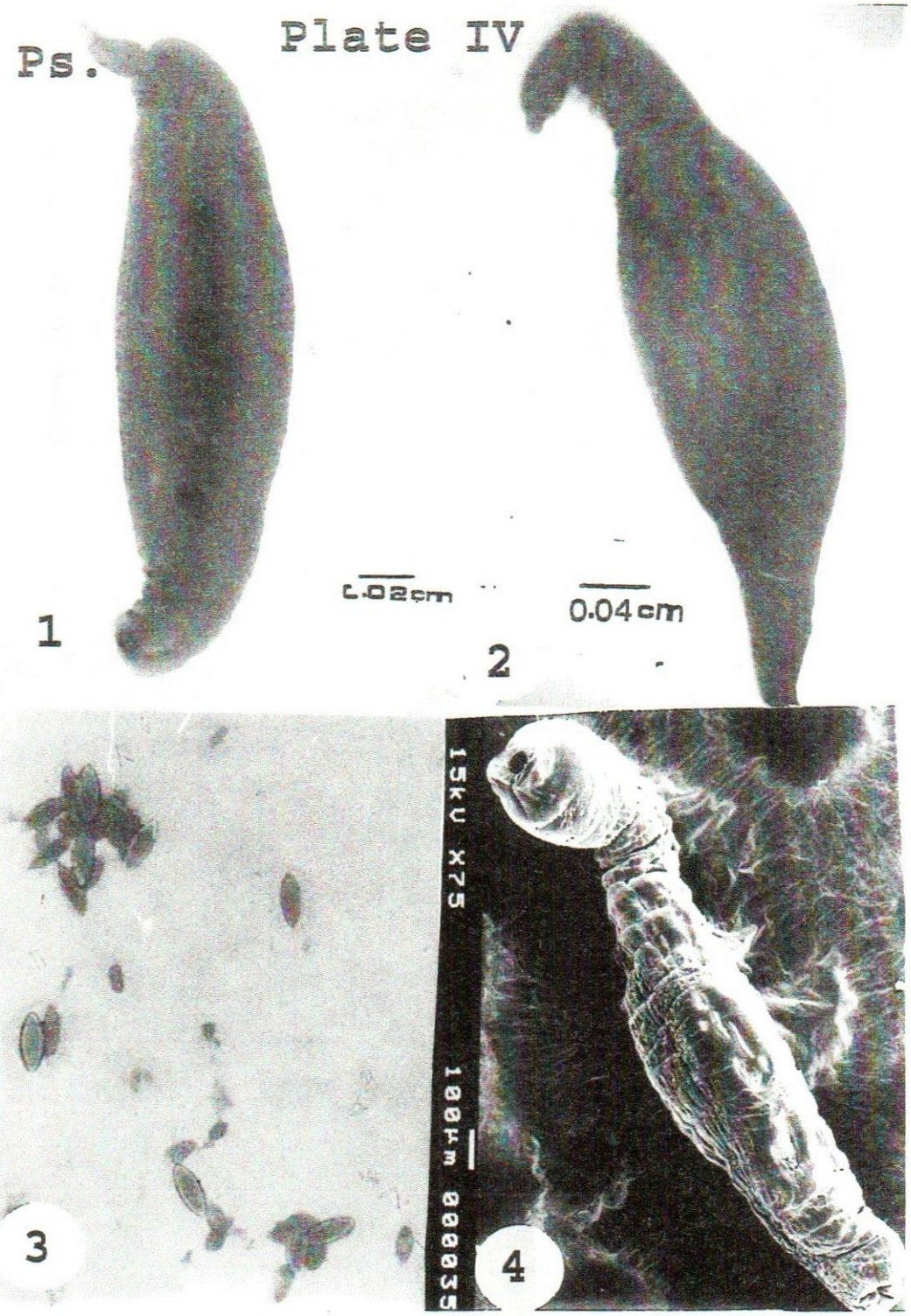
Plate IV



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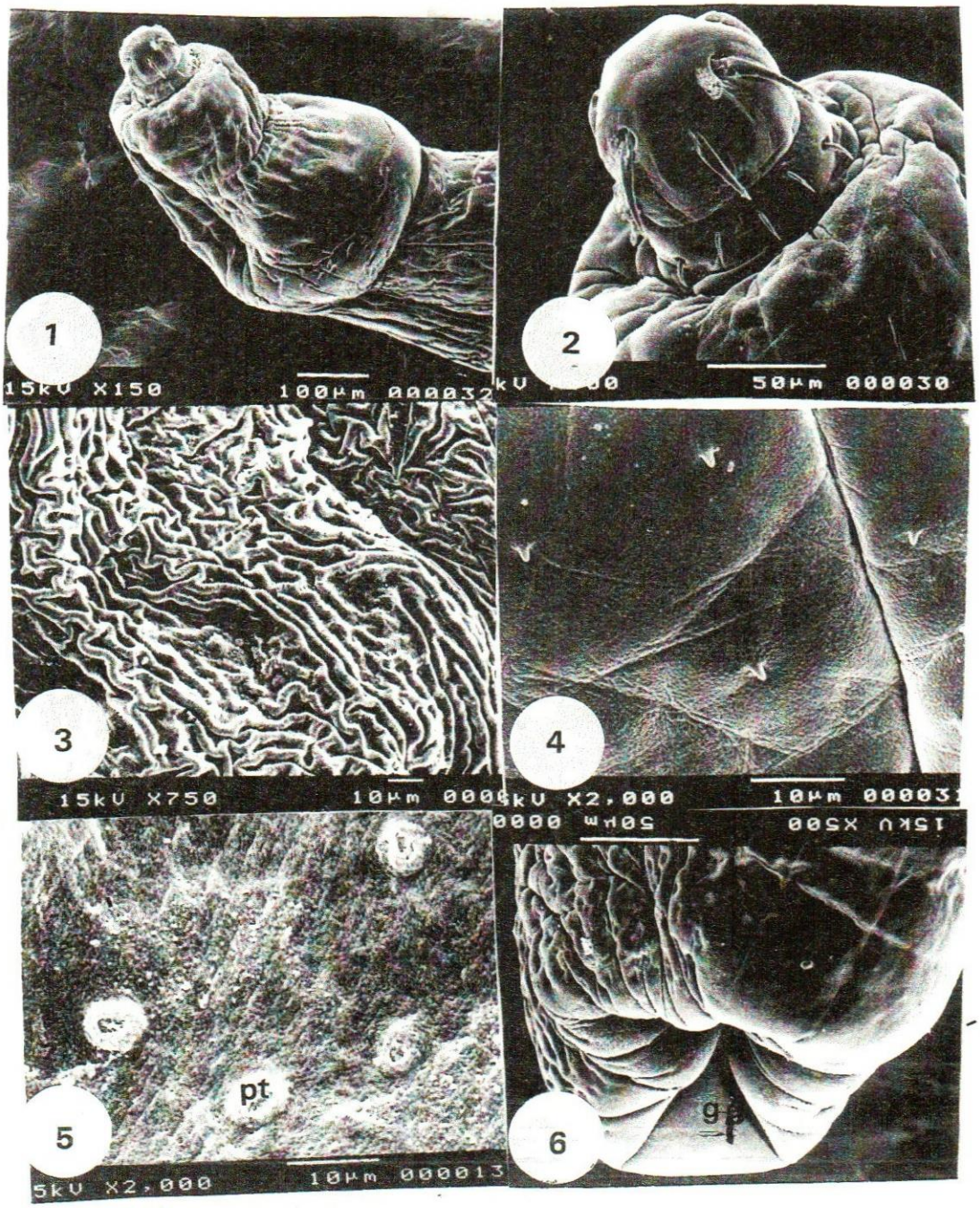
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## EXPLANATION OF FIGUERS

Light microscope of *Procamallanus laeviconchus and Rhadinorhychus niloticus.*

**Plate I:**

**Fig. 1:** Anterior end of *Procamallanus laeviconchus* showing buccal capsule X100.

**Fig. 2:**Posterior end of male *Procamallanus laeviconchus* showing *spicule X100.*

**Fig. 3:** Posterior end of female *Procamallanus laeviconchus* showing anus X100.

**Fig. 4 :** Photomicrograph of male *Rhadinorhychus niloticus.*

**Fig. 5 :** Photomicrograph of female *Rhadinorhychus niloticus.*

**Fig. 6:** Photomicrograph of Proboscis *Rhadinorhychus niloticus* X50.

**Fig. 7:** Photomicrograph of the egg of *Rhadinorhychus niloticus* X400.

**SEM of the anterior end of *Procamallanus laeviconchus.***

**Plate II:**

**Fig. 1:** Lateral view of mouth opening.

**Fig. 2:** Top view showing cephalic papillae.

**Fig. 3:** Top view showing Saw-like teeth and the bottom of the buccal capsule.

**Fig. 4:** Showing deirid (d).

**Fig. 5:** Top view showing caudal papillae in male.

**Fig. 6 :** Lateral view showing cloaca (c)and caudal papillae (P) in male.

**Fig. 7:** SEM micrograph of the lateral view of the posterior end of male showing caudal papillae.

**Fig. 8:** SEM micrograph showing transverse striations of the cuticle.

**SEM of *Rhadinorhynchus niloticus***

**Plate III:**

**Fig. 1:** Proboscis and anterior part of the trunk.

**Fig. 2&3:** Magnification of Proboscis hooks.

**Fig. 4:** Showing trunk spines of *Rhadinorhynchus niloticus* .

**Fig. 5:** Showing transverse striations on the trunk region.

**Fig. 6:** Showing posterior end of the worm and genital pore (g.p.)

**Light microscope of *Acanthogyrus (Acanthosintis) tilapiae***

**Plate IV:**

**Fig. 1 :** Photomicrograph of male.

**Fig. 2 :** Photomicrograph of female.

**Fig. 3 :** Photomicrograph of eggs X400.

**Fig. 4 :** SEM of *Acanthogyrus (Acanthosentis).*

**SEM of *Acanthosentis (Acanthosentis) tilapiae***
**Plate V :**
**Fig. 1:** Proboscis and trunk region.
**Fig. 2:** Magnification of proboscis and hooks.
**Fig. 3:** Showing the wrinkles of the trunk region.
**Fig. 4:** Magnification of trunk spines.
**Fig. 5:** Showing Amoeboid structures (cuticular pits)of the body surface.
**Fig.6 :** Posterior end of genital pore (gp).

## DISCUSSION

*Procamallanus* comprises numerous species described from fresh water and marine fishes. Many authors consider forms with spiral ridges in the buccal capsule to represent a separate genus *Spirocamallanus* Olsen, 1952. From comparison of, the present material *Procamallanus laeviconchus* with specimens of Imam (1971), Al-Bassel (1990) and El-Ganiny (1995), it was found that both males and females of the present parasite are shorter than their specimens. The present males have two unequal spicules, the shorter spicule is hardly visible while the longer one is longer than their specimens. Moreover minor differences in measurements of buccal capsule, muscular and glandular oesophagus, distance to nerve ring and to vulva from anterior end were also observed.

As compared to the *P. laeviconchus* specimens from *Clarias gariepinus* studied by Moravec (1974, 1975) in Egypt, specimens of the present material from *Synodontis* spp. show some differences, mainly in the number and arrangement of caudal papillae and the preequatorial situation of vulva in the gravid female .

SEM showed detailed structures particularly in the anterior end and caudal end of the male in the form of 6 anterior lips, 4 submedian cephalic papillae, minute saw-like teeth at the bottom of the buccal capsule and 8 blunt caudal papillae. These might be of great importance in differentiation of different *procamallanus* spp.

Acccording to Yamaguti (1958), the present first acanthocephalan parasite belongs to genus *Rhadinorhychus* Luhe, 1911 which is related to family Rhadinorhynchidae Travassos, 1923 the characters of the present acanthocephalon worms coincide with those of *Rhadinorhynchus niloticus* encountered and described by Mohamadain (1989) for the first time in Egypt from the intestine of *Lates niloticus* and *Bagrus bayad* from River Nile at Qena province. Compared with previous species, we noticed that there are common characters between the present parasite and these specimens but, with minor differences in

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