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# Light and Electron microscopic Studies of *Parapharyngodon sceleratus* and *Physaloptera* sp. Discovered in Some Reptiles of Qena Governorate, Egypt

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**Abstract:** ***The present study is the first that has been carried out on redescription of helminth parasites infecting reptiles in Qena Governorate. Light microscope, scanning electro microscope and Camera Lucida were used. During the present study about 106 individuals of Chalcides ocellatus (eye shrink or Sehliadoffana) and 24 individuals Psammophis sibilans (a snake) were collected from Qena Governorate. Two nematode species were collected and identified. Parpharyngodon sceleratus (Physalopteridae, Travassos, 1919) and Physaloptera sp. (family: Physalopteridae, Leiper 1908). The first and the second species were collected from the larg intestine of Chalcides ocellatus and stomach of Psammophis sibilans, respectively. In relation to P. sceleratus, the prevalence rate of infection was 4.9%, while it was 8.3 % in case of Physaloptera sp.***

**Keywords:** Light and electron microscope - *Paraharyngodon scleratus- Physaloptera* sp. - reptiles – Qena Governorate-.Egypt.

## 1. Introduction

Reptiles are most abundant in the warmer regions of the world and occupy different habitats. Reptiles have been established as a significant source of diseases in humans for several decades.

Several authors described many helminth parasites collected from some species of reptiles. Of them, Elwasila (1990) who isolated *Physalopteroides tarentolae* n. sp. from the gecko, *Tarentola annularis* and could make description. Moreover, Ashour *et al*., (1994) described collected oxyurid nematode *Parapharyngodon bulbosus* from the lizard, *Chalcides ocellatus*.

In relation to the pharyngodoniid parasites, they were isolated and depicted by Bursey and Goldberg (1996 b&c) who worked on *Gehyra oceanic* and *Hemidactylus frenatus*, respectively. However, Goldberg *et al.*, (1999a, b &c) described two species of the nematodes *Physaloptera retusa* larvae and *Spauligodon giganticus* and other nematode parasites collected from side-blotched lizards, *Utastans buriana*, the *Madrean alligator* lizard, twenty day geckos and the lizard *Barisia imbricate* harbored, respectively. In (2001a& b), Goldberg and Bursey dissected gekonid lizards and *Mabuya* for collectingand description of two and five nematodes. On Bursey and Goldberg (2005), they described two new species of pharyngodoniid parasite *Parapharyngodon kenyaensis* and *Thelandross amburuensis.* However, Bursey and Goldberg (2007a&b) get and described the pharyngodoniid parasite, *Parapharyngodon baueri*, from legless skink, *Typhlosaurus lineatus* as well as the pharyngodoniid parasite *Parapharygodon grismeri* from the rock lizards *Petrosaurus repens*, and *Petrosaurust halassinus*. Recently in 2013 Goldberg *et al.* showed hard efforts for characterization eight species of nematode: gravid specimens of *Cosmocercaparva*, *Parapharyngodon scleratus*, *Physaloptera retusa*, *Skrjabine laziagalliardi*, *Spauligodon bonairensis* and *Spauligodon oxkutzcabiensis*, larvae of *Physaloptera* sp. and *Acuariidae* gen. sp. Furthermore, some helminth pharyngodonid parasites were isolated, examined and described such as *Mesocoelium danforthi*, *Spauligodon analis* and *Parapharyngodon cubensis* as Dyer *et al.*, (2001).

The pharyngodoniid parasite *Parapharyngodon riojensis*, was studied by Ramallo *et al.*, (2002b), while the parasite *Parapharyngodon echinatus*, collected from from the Senegal gecko, *Tarentola parvicarinata*. was examined via morphometric and molecular characterization as Mašová *et al.*, (2008).

Yildirimhan *et al.*, (2009) found two nematode species *(Parapharyngodon micipsae* and *Thelasto matoid* nematode (Nemathelminthes). McAllister *et al.*, (2010b) described 1 atractid,1 diaphano cephalid, 1 heterakid, 3 pharyngodonids, and 2 physalopterans. Pereira *et al.*, (2011) described the pharyngodon nematode, *Parapharyngodon bainae* n. sp. Albuquerque *et al.*, (2012) recovered the nematodes *Physaloptera lutzi*, *Physaloptera retusa* and *Physalopteroides venancioi*, from lizards. Pereira*et al.*, (2012) showed a description on *Physaloptera tupinambae* n. sp. (Nematoda: Physalopteridae) from *Tupinambis merianae* (Squamata: Teiidae). Roca *et al.*, (2012) described two nematodes, *Parapharyngodon micipsae* (Seurat, 1917) and *Pharyngodonidae* gen. sp. from chalcides.

The aim of this study is constructed to redescribe some nematodes infecting some important reptiles in Qena Province. Therefore, the studied nematode species were identified in the Zoology Department Faculty of Science, Cairo University.


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## 2. Materials and Methods

Collection of helminthes: The collected hosts were dissected. The oral and body cavity were examined. The general viscera was removed and placed in physiological saline solution (0.7%). The parasites were removed and washed with saline solution to remove the adherent debris.

**2.1 Preparation of helminthes for light microscopically examination**:

1) Fixation: The nematode parasites were fixed in hot 70% ethyl alcohol to be sure of their relaxation.
2) Preservation: The worms were preserved in 70% ethyl alcohol mixed with 10% glycerol to avoid sudden drying.
3) Examination: For microscopic examination and identification, the nematodes were mounted on a slide with few drops of lacto phenol. The former technique was used to help in description and counting cervical and caudal papillae.

**2.2 Preparation of samples for electron microscopically examination**

For scanning electron microscopy, some of freshly obtained parasites were fixed in cold 5%glutraldhde in 0.1 M cacodylate buffer for 24 hours. The material was washed in 0.1 M sodium cacodylate buffer (PH 7.4) three times, 15 minutes for each and then treated with osmic acid for 2 hours. Washing three times with buffer was repeated and then the material was dehydrated through aggraded ethanol series and then dried at 30°c for 30 minutes. After complete drying, the material was mounted on holders with silver paint and coated with a layer of gold under vacuum. Specimens were investigated by using JEOL JSM-5400 LV Scanning Electron Microscope at 15 K volt. Electron micrographs were taken with different magnifications and analyzed.

**2.3 Drawing, Measurements, Photomicrography and Electron micrograph of helminthes**

Carl Zeiss drawing camera Lucida was used for drawing the specimens. Calibrated eye piece was used for measuring the specimens. For all micrographs Zeiss photo research microscope and JEOL JSM-5400 LV Scanning Electron Microscope were used.

**Identification**: The collected parasites were identified by Prof. Thoraia Elassly, Prof. of Parasitology, Parasitology Department, Animal Health, Re. Inst.

## 3. Results and Discussion

***1-Parapharyngodon sceleratus***

- Order : Ascarididae Skrjabinet Schulz, 1938
- Suborder : Oxyurata Skrjabin, 1923
- Superfamily : Oxyuroidea Railliet, 1916
- Family : Pharyngodonidae Travassos, 1919
- Genus : *Parapharyngodon* Chatterji, 1933
- Species : Scleratus Travassos,1923

The present nematod parasite was collected from the large intestine of *Chalcides ocellatus* 10out of 106 were found infected and the prevalence of infection was 9.4%. All of the infected hosts represented a double infection Worms tapering at both ends, body cuticle transversally striated. Mouth opening is surrounded by six small lips.

**3.1 Male**

The body length is 1.8-2.3 mm and the maximum width 0.1-0.14 mm (Plate 1a), oesophagus length 0.40-0.50 mm from anterior of the body (Fig. 1A & plate 1b). The distance of excretory pore from the anterior of the body 0.80–0.85 mm. Tail reduced to slim appendage inserted dorsally, directed obliquely to longitudinal axis of body, the tail length 0.02–0.06 mm (Fig. 1B & plate 1c). There are nine caudal papillae (Plate 2d).

**3.2 Female**

The female body is 1.9-2.5 mm long and 0.11-0.23 mm maximum width. Oesophagus length is 0.42-0.58 mm and leads to intestine (Fig. 1C & plate 1d). Vulva is 0.27-0.60 mm from anterior extremely end; tail is 0.05-0.19 mm long (Fig. 1E & Plate 2e). The egg is oval in shape (Fig. 1D & plate 1f).

**3.3 Scanning Electron Microscopically study**:

The body is transversely striated and this striation of cuticle is beginning just below the cephalic collaret. In the female the mouth is terminal in position and surrounded by three bilobed papillae; one is dorsal and two ventro-lateral (Plate 2 a-c). In the male there are nine caudal papillae on the posterior end of body (plate 2d).

## 4. Discussion

Baylis (1936) and Petter & Quentin (1976) considered *Parapharyngodon* a synonym of *Thelandros*, however, male genital cone of *Thelandros* is prominent. The posterior lip of the anus is drawn out into a process supported by a v-shaped sclerotized accessory piece; the caudal appendage is, therefore, inserted sub terminally on the body. In males of *Parapharyngodon*, the genital cone is only slightly developed or absent and the posterior anal lip lacks an accessory piece; the caudal appendage is inserted terminally. The tail in the female in *Parapharyngodon* is rounded and terminates in a short conical appendage which is often curved dorsally. In *Thelandros* the female tail is variable. In some species it is conical, tapering evenly from the anus while in others it is rounded and supports a short filiform appendage. Eggs of *Thelandros* are larvated in utero and the operculum, when present, is polar in position. Eggs of *Parapharyngodon* are deposited in an early stage of cleavage and the operculum is sub terminal. *Parapharyngodon* spp. is parasite of omnivorous or herbivorous hosts.

Read *et al.*, (1952) distinguished *Pseudothelandros* from *Thelandros* by the presence of lateral alae in males of the former.


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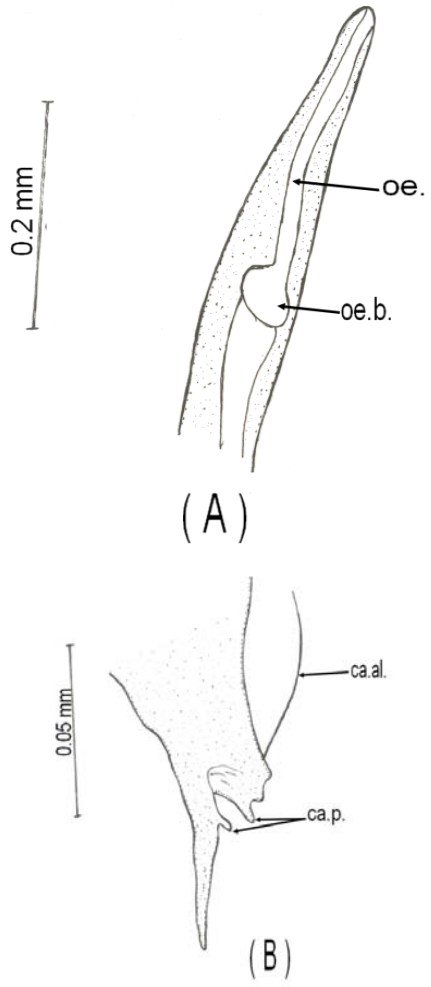
Freitas (1957a) pointed out, however, that *Parapharyngodon* was distinguished from *Thelandros* on the same basis and reduced *Pseudothelandros* to synonymy with *Parapharyngodon*.

Travassos (1923) included no illustrations in his original description. The species has been redescribed from material collected from *Tropidurus toruatus, Tapinuruss cutipunctatus, Hemidactylus mabouia* and *Ameivaameiva.*

*Parapharyngodon scleratus* shows similarities to *P. riojensis* and *P. lamothei,* but it differs as follows:

*P. scleratus*has has a longer spicule than *P. lamothei* and an additional pair of sublateral papillae.

- Female of *P. scleratus* has spike-shaped tails.



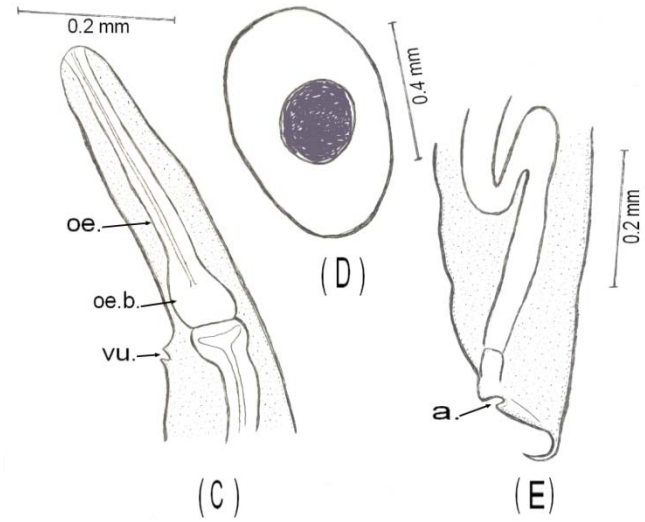
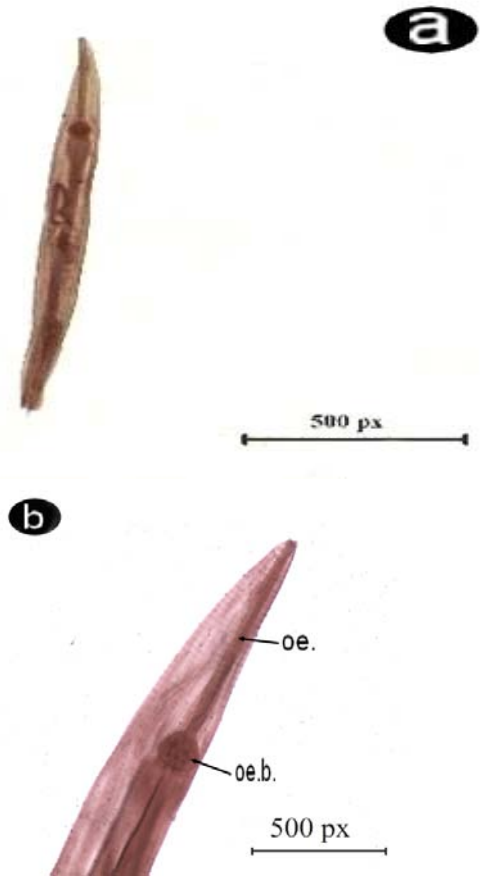




**Figure 1** :***Parapharyngodon scleratus***: A) male, anterior end; (B) male, posterior end; (C) female, anterior end; (D) egg; (E) female, posterior end. (oe.= oesophagus, oe.b.= oesophageal bulb, vu.= vulva, and a.= anus.





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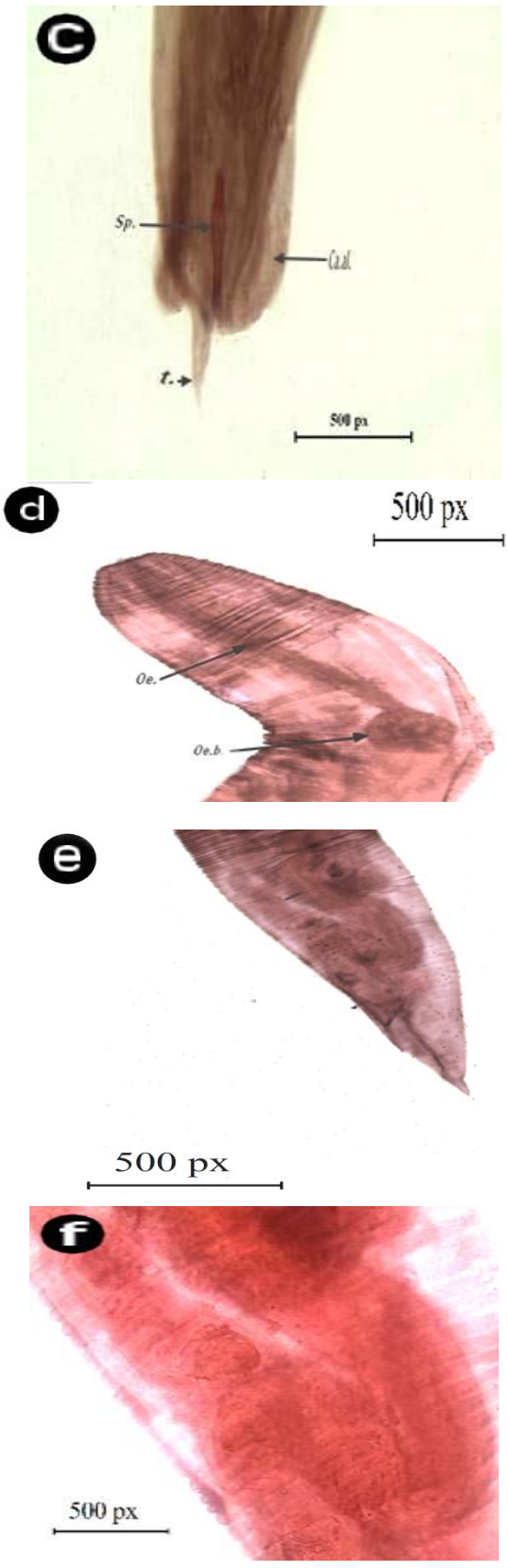
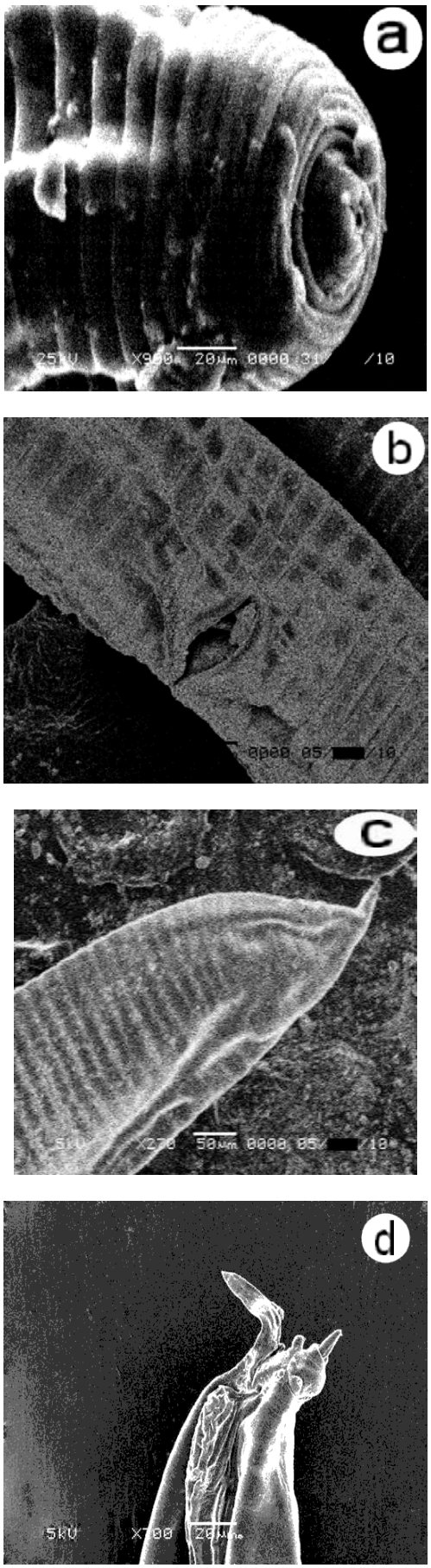



**Plate 1:** *Parapharyngodon scleratus*:a) male, The entire male. b) male, Anterior end.c) Posterior end. d) female, Anterior end. e) female, Posterior end. f) The egg . (oe. = oesophagus and oe.b.= oesophageal bulb.




**Plate(2):** *Parapharyngodon scleratus*: Scanning Electron Microscope a) female, The anterior end (face view). b) female, vulva opening. c) female, Posterior end. d) posterior end of male shows caudal papillae.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*2- Physaloptera* sp.
Order :Spiruridea Diesing 1861
Family :Physalopteridae Leiper 1908
Genus :*Physaloptera* Rud 1819

The present nematode parasite was collected from the stomach of *Psammophis sibilans* 2 out of 24 were found infected and the prevalence of infection was 8.3%. This parasite is white in color with long cylindrical body. Mouth is surrounded by two large and lateral lips; each lip is armed with a variable number of teeth and carrying two external papillae. The mouth is provided with a large cephalic collaret .Oesophagus consists of an anterior muscular and a posterior glandular part .

**Male**
The length of the body is 10.4-13.2 mm and the width is 0.32-0.49 mm. Mouth is surrounded by large simple triangular lateral lips, each is armed with two teeth; with a large cephalic collaret (Fig. 2A& plate 3a), the mouth leads into tubular oesophagus, the length of oesophagus is 1.7-2.1 mm and leads into the intestine. The distance of testes from the anterior end of the body is 1.95-1.05 mm. There is a large caudal alae which supported by a long cost form papillae (Fig. 2B& plate 3b). Caudal alae are fused anteriorly.

**Female**
The length of the body is 10.30–11.75 mm, and the width is 0.28-0.39 mm. The oesophagus leads into the intestine (Fig. 2C & plate 3c). The uterus occupies most of the body (plate 3d). The vulva is situated in the anterior half of the body. The eggs are oval in shape; smooth, thick–shelled and embryonated when laid (Fig. 2E&plate 3e).

**Scanning Electron Microscope:**
The mouth is provided with a large simple triangular lateral lips armed with two teeth (Plate 4a); the mouth with a large cephalic collaret (Plate 4b). Plate 4( c-d) show the posterior end of female.

## 5. Discussion

Adult nematodes of the genus *Physaloptera* (Rudolphi, 1819), had been found in the stomach and seldom in the small intestine of amphibians, reptiles, birds and a wide range of insectivorous and omnivorous mammals throughout the world.

The first record of *Physaloptera marsupial* was that of Ortlepp 1922, who described a species from a long-nosed bandicoot *Peramelesnasuta* Geoffroy, which died at the London Zoo. Ortlepp did not name the species, because the ventral caudal papillae on tail of the male were asymmetry abnormal.

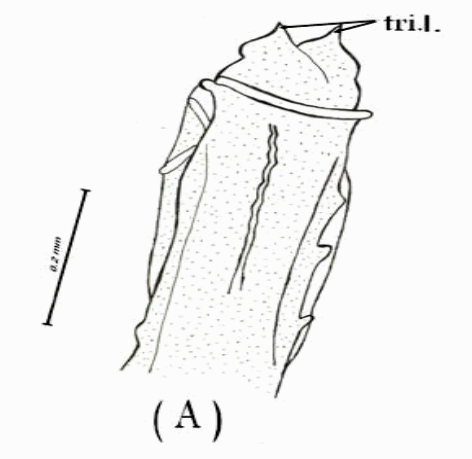
In 1999, Norman and Beveridge redescribed the *Physaloptera* species (*P. peramelis*, *P. thalacomys*, and *Physaloptera* sp. from both *Peramelesnas uta* and *Isoodonmacrourus*) in bandicoots (Marsupialia: Perameloidea) in Australia.

The parasite is usually firmly attached to the mucosa of the definitive host, from where they feed on blood. It has been reported that the parasites may occasionally change their site of attachment, therefore causing the formation of numerous small oedematous ulcers, and consequently bleeding, inflammation and increased mucus production. Heavy infections lead to anemia, weakness, anorexia, diarrhea, cachexia, and weight loss. Diagnosis often relies on the observation of clinical signs and parasite eggs in the faces of the vertebrate host.

In 1923, Travassos separated four new genera from the genus *Physaloptera*, limiting the genus *Physaloptera* to those forms possessing similar and subequal spicules, two uteri, four pairs of pedunculaed papillae, and having no reduplication of the cuticle over the caudal extremity. The present author gives the following key to these five and three other nearly related genera:

1- Spicules similar and subequal.
A- Two uteri:
a- Four pairs of pedunculated papillae; no prepuce-like sheath at the poserior extremity……*Physaloptera.*
b- A prepuce-like collar presents at the posterior extremity ….*Chlamydonema.*
c- Eight pairs of pednculated papillae ….……*Thubunaa.*
d- Ten uteri ……..…….. *Turgida.*

2- Spicules dissimilar, and their sizes are very different.
AA- Two uteri.
aa – Four pairs of pedunculated papillae ……*Abreniata.*
bb - Nine to ten pairs of pedunculated papillae
cc- Vulva in anterior half; ovi jector very long ……..…… *Heliconema.*
dd- Vulva near to the anus…….. *Proleptus.*
BB- Four uteri ……..……..... *Leptosoma*





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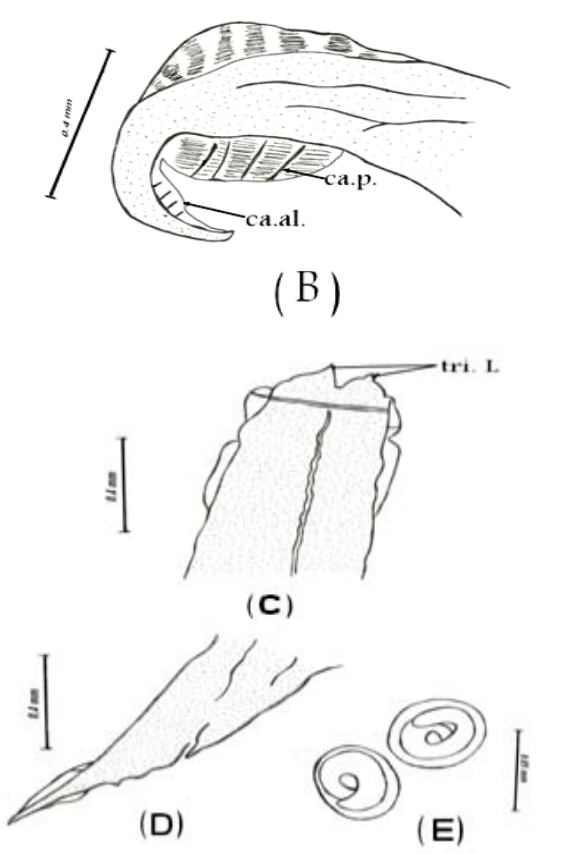


**Figure 2:** *Physaloptera* sp.A) male, Anterior end, B) male, Posterior end, C) female, Anterior end, D) female, Posterior end, E) The egg (tri.l.= triangular lips, ca.al.= caudal alae and ca.p.= caudal papillae).

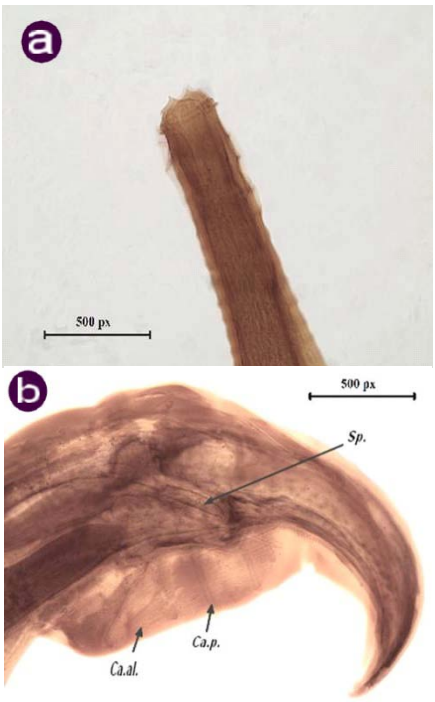



Plate (3): *Physaloptera* sp. a) male, anterior end, b) male, posterior end c) female, anterior end. d) female, posterior end. e) The egg. (ca.al= caudal alae, ca.p= caudal papillae and sp.= spicule.


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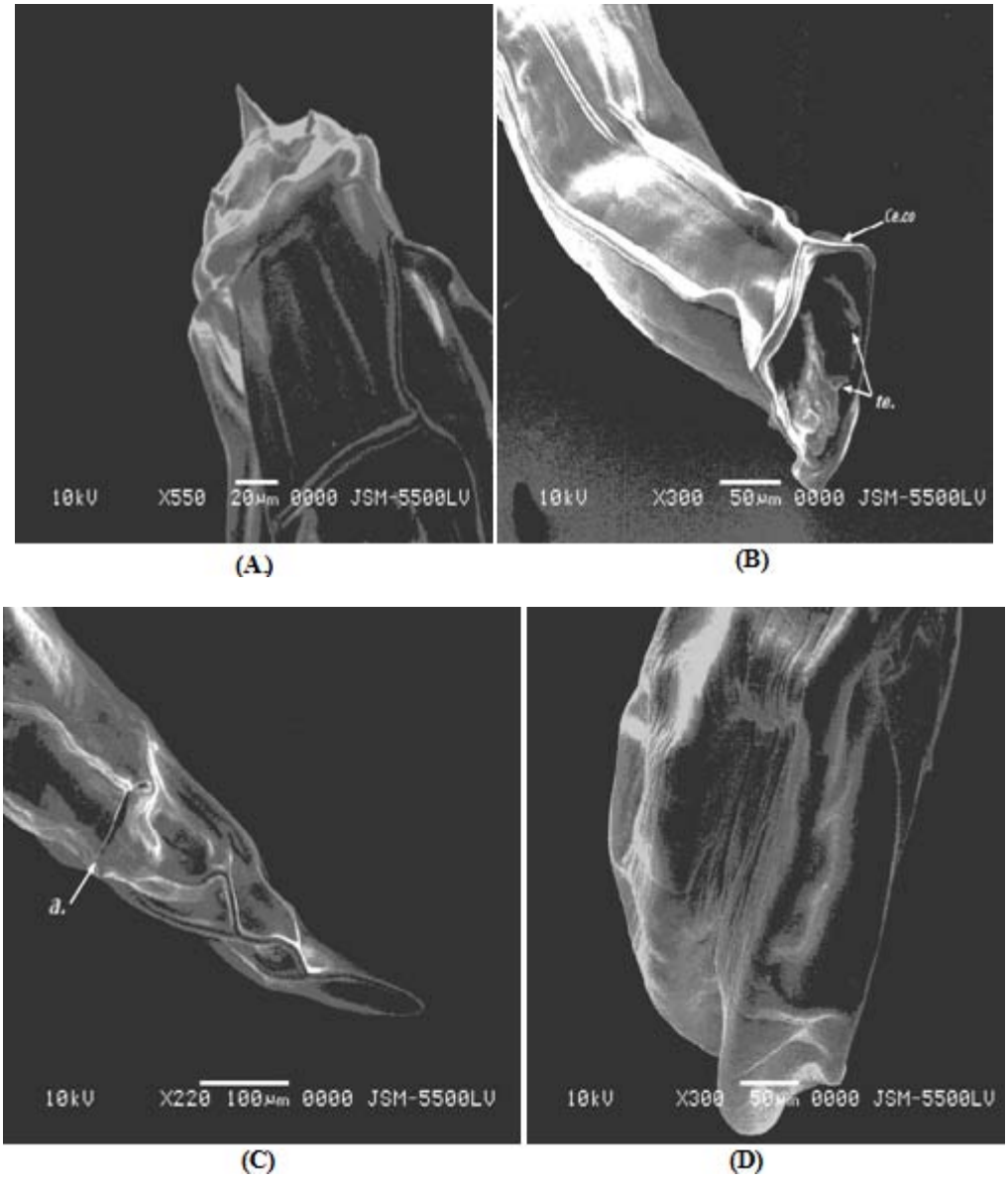


**Plate (4):** *Physaloptera* sp.Scanning electron microscope showing: A) Anterior end of the parasite (dorsal view), B) Anterior end of the parasite (face view). C and D Posterior end of female. (ce.co = cephalic collerate , te. = teeth and a.= anus ).

## 6. Acknowledgment

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