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MORPHOLOGICAL AND ULTRASTRUCTURAL STUDY ON TYPE I AND TYPE II THIRD STAGE LARVAE OF *ANISAKIS SIMPLEX* FROM RED SEA FISHES FROM HURGHADA, EGYPT

By

REFAAT MA KHALIFA[1], ABDEL-NASSER A. HUSSEIN[2], NERMEAN M. HUSSEIN[2] and **ZEINAB T. M. ABDEL-GHAFFAR[2]**

Department of Medical Parasitology[1], Faculty of Medicine, Assiut University, and Department of Zoology, Faculty of Science[2], South Valley University, Qena Governorate, Egypt (*Correspondence:rkhalifa_eg@yahoo.com)

Abstract

Study of the prevalence and identification of the juveniles of *Anisaxis* spp. in the marine fishes is of great value for biologists and medical parasitologists. In this study, Red Sea fishes at Hurghada, Egypt were examined for adult and larval helminthes. Among others, *Anisakis simplex* third stage larvae; type I & type II were identified from three fish hosts; *Caranx sexfasciatus*, *Cephalopholis miniata* and *Variola louti*. The prevalence of larvae was reported and their light and SEM morphological characters were illustrated. The encountered larvae were compared with previously described ones from Red Sea fishes from Egypt, Yemen and Arabia Saudi, and their zoonotic pathogenesis was discussed.

Key words: Red Sea fish, *Anisakis simplex*, Type I, Type II, 3rd stage larvae, Hurghada, SEM.

Introduction

Anisakis spp. larvae are of worldwide distribution and are commonly found in flesh and body cavity of many species of marine fishes (Tantanasi *et al*, 2012). Juveniles of different anisakid species were previously described from Egypt; from the Red Sea fishes (Abdou, 2005; Abdou and Dronen, 2007; Arafa *et al*, 2009; Morsy *et al*, 2015; Abou-Rahma *et al.*, 2016) and from the Mediterranean Sea at Alexandria (Mohamed and Abd El-Ghany, 2011; Abou Zaid *et al*, 2018) and from other coasts of the Red Sea in Yemen (Al-Zubaidy, 2010; Al-Zubaidy *et al*, 2012) and from KSA (Hassan *et al*, 2013; Ibrahim *et al*, 2018).

The aim of the present work was to identify and describe different types of *Anisakis simplex* in Red Sea fishes at Hurghada.

Material and Methods

Fishes were transported as alive as possible to the Parasitology laboratory, Zoology Department, Faculty of Science, South Valley University. Fishes were identified according to criteria of (Randall, 1983; Lieske and Myers, 2004; Lieske *et al*, 2004) and more confirmed through the fish base (website http:// www. fishbase.org). The gastrointestinal tract was untangled with fingers (Justine *et al*, 2012). Whole digestive system and other viscera were opened longitudinally. Macroscopic and microscopic examination of different organs was carried out for detection of any visible nematode larval parasites. The collected larvae were cleaned by washing several times with isotonic saline solution. The relaxed larvae were preserved in bottles containing mixture of 70% alcohol and 5% glycerin. For microscopical examination the larvae were mounted on slides with few drops of lactophenol and covered by a cover slip. Identification of the encountered larvae was done according to the keys of the nematode parasites of vertebrates (Yorke *et al*, 1926; Yamaguti, 1963).

Results

Incidence: Out of four *Caranx sexfasciatus*, six *Cephalopholis miniata* and one *Variola louti* Red Sea fishes examined, two, two and one specimen respectively were found harboring type I & type II of the third larval stage of *Anisakis simplex* in their small intestine (worm burden 1-4 per fish host).

Morphology of *A. simplex* type I 3rd stage larva based on one larva specimen. (Pls. 1, 2 & 3): Body is white, slender in shape and measures 13mm in length by 0.540 mm in width. Cuticle is characterized by annular

striations covering the surface of the body with longitudinal annulations, whereas tail region has transverse striations and longitudinal annulations. Mouth is triangular anteroventrally with a ventral boring tooth, located ventral to mouth and three inconspicuous lips surrounding mouth. Also, four small papillae (two dorsolateral & two ventro-lateral) surrounding the mouth opening. Esophagus is cylindrical, muscular, measures 1.050mm in length x126µm width. Ventriculus is an oblong junction with intestine; measures 459µm in length by 164µm in width. Distance of nerve ring from anterior extremity is 165 µm, excretory pore as a transverse slit between ventrolateral lips & opens at a single excretory duct. Rectum opens ventrally at anus, surrounded by rectal glands. Tail is conical, with a terminal mucron, measures 132µm length. Mucron length is 16µm.

Anisakis simplex type II 3rd stage larva based on 5 larvae specimens (Plates 4-5 & 6)**:** They were found free in the intestine and encapsulated (coiled in a thin walled cyst) on wall of intestine. Body is brownish, thickest posteriorly, tapering gradually towards anterior end; length is 10.88-14.16 (12) mm by width is 0.208-0.398 (0.272) mm. Cuticle is characterized by fine transverse striations, irregularly wrinkled near tail. Mouth is triangular anteroventrally with a ventral boring tooth measuring 6-8(7) µm in length, located ventral to mouth projecting anteroventrally and three small lips surrounding mouth. Esophagus cylindrical, muscular, measures 0.829-1.1.00 (0.900)mm in length x 80-114 (94)µm in width. Ventriculus is a horizontal junction with intestine, measures 386-586 (480)µm in length x 100-119 (107)µm in width. Distance of nerve ring from anterior extremity is 200-352 (275)µm. Excretory pore, as a transverse slit between ventrolateral lips and opens at a single excretory duct. Rectum opens ventrally at anus and surrounded by rectal glands. Tail is conical, without a terminal mucron, measures 142- 205 (170) µm length.

Ultrastructure: *A. simplex* 3rd stage lar-va type I: Anterior end was provided with three prominent lips, one dorsal and two ventrolateral surrounding the triangular mouth opening. Four small cephalic papillae: two at the dorsal lip and two at the ventrolateral lip. Papillae were oval, flat and well demarcated by a shallow groo-ve. Prominent boring tooth was triangular in shape and directed outwards. Excretory pore open ventrally at anterior end and located between bases of subventral lips. Cuticle is provided with longitudinal annulations due to fine grooves and annular striations plus body papillae are noted. Both transverse and longitudinal striations were located in posterior extremity; transverse striations were due to various depths and width. Posterior extremity was conical shape and tail provided with terminal mucron.

A. simplex 3rd stage larva type II: Encapsulated larvae were coiled comma shaped in a thin walled cyst. A prominent boring tooth began to appear in anterior end.

Table 1: *Anisakis simplex* (3rd stage larva, type I, type II (all measurements in µm unless otherwise mentioned

Reference	Morsy *et al.* (2015)	Present study
Fish host(s)	*Saurida undosquamis*	*Caranx sexfasciatus*
Locality	Hurghada, Egypt in Red Sea	Hurghada, Egypt in Red Sea
Site of infection	Peritoneal cavity around wall of stomach as encapsulated larvae	free larvae in intestine and encapsulated larvae around wall of intestine
Body length	18±2 (16-20) mm	10.88-14.16 (12) mm
Maximum body width	0.48 ± 0.2 (0.28-0.68) mm	0.208-0.398 (0.272) mm
Boring tooth	0.005±0.002 mm	0.006–0.008 (0.007) mm
Esophagus length	1.38±0.02 (1.36-1.4) mm	0.829-1.100 0.9 mm
Esophagus width	0.12±0.02 (0.1-0.14) mm	0.80-0.114 (0.94) mm
Tail length	0.2±0.02 (0.18-0.22)	0.142-0.205 (0.170) mm

Table 2: Comparison between *Anisakis simplex* (3rd stage larva, type I) of present specimen and previously described forms (all measurements in µm unless mentioned in mm).

Reference	Type I	Type II
Fish host(S)	*Caranx sexfasciatus*	*Caranex sexasciatus, Cephalopholis miniata & Variola louti*
Body length	13 mm	10.88-14.16 (12) mm
Maximum body width	0.540 mm	0.208-0.398 (0.272) mm
Esophagus length	1.05 mm	0.829-1.1.00 (0.9) mm
Esophagus width	126	80-114 (94)
Ventriculus size	459x164	386-586 (480)x100-119 (107)
Nerve ring to anterior end	165	200-352 (275)
Tail length	132	142-205 (170)
Terminal mucron	Present; 16	Absent

Table 3:Comparison between previously described *Anisakis simplex* (3rd stage larva, type II) and present specimen (all measurements are in µm unless mentioned in mm).

Reference	Morsy *et al.* (2015)	Present study
Fish host(s)	*Saurida undosquamis*	*Caranx sexfasciatus*
Locality	Hurghada, Egypt in Red Sea	Hurghada, Egypt in Red Sea
Site of infection	Peritoneal cavity around stomach wall as encapsulated larvae	free larvae in intestine and encapsulated larvae around intestine wall
Body length	18±2 (16-20) mm	10.88-14.16 (12) mm
Maximum body width	0.48 ± 0.2 (0.28-0.68) mm	0.208-0.398 (0.272) mm
Boring tooth	0.005±0.002 mm	0.006–0.008 (0.007) mm
Esophagus length	1.38±0.02 (1.36-1.4) mm	0.829-1.100 0.9 mm
Esophagus width	0.12±0.02 (0.1-0.14) mm	0.80-0.114 (0.94) mm
Tail length	0.2±0.02 (0.18-0.22)	0.142-0.205 (0.170) mm

Discussion

Specimens were identified as belonging to Superfamily Ascaridoidea Baird, 1853 after Yorke *et al.* (1926), Family Anisakidae Railliet & Henry, 1912 after Arai and Smith (2016) and genus *Anisakis* Dujardin, 1845 possessing all characteristics of that genus (inconspicuous lips with a prominent boring tooth on anterior end; a straight anterior gut consisting of esophagus, ventriculus, and intestine; a cuticle obviously transversely striated, and larvae encysting in capsules of the host origin (Rocka, 2004; Dixon, 2006). Identification was based on comparison between morphological characteristics of larval types of anisakids by digestive tract, shape and presence of boring tooth or lips at anterior end, position of excretory pore and shape of post anal tail together with presence or absence of a typical terminal mucron (Anderson, 1992; 2000; Shih and Jeng, 2002; Rocka, 2004; Al-Zubaidy, 2010).

Nematodes from Superfamily Ascarididea (Families: Anisakidea and Raphidascarididea) commonly named anisakids, are parasites of many water organisms, low specificity in choice of hosts, both intermediate and definitive, hence; their wide geographical distribution (Szostakawska *et al,* 2005). Fish act as intermediate or paratenic host, whereas marine mammals, definitive hosts, harbor adult stages (Anderson, 1992).

Infective third stage larvae of *Anisakis* are common in commercially imported marine fishes and its presence is of great concern for public health and economic reasons (Choi *et al*, 2011; Tantanasi *et al*, 2012; Bilska-Zagac *et al*, 2015).

Shape of head region and tail with presence or absence of a tail mucron distinguished between *Anisakis* Type I & II, first type is characterized by an anterior head with dorsal and ventrolateral lips equipped with papillae and postanal region terminated at a mucron, while *Anisakis* Type II is characterized by a head region surrounded by four papillae with no lips; postanal tail is rounded without a terminal mucron **(**Shukhgalter and Nigmatullin, 2001; Pardo-Gandarillas *et al*, 2009).

The present larvae were identified as belonging to *A. simplex* type I & type II third

stage larvae as they were more or less similar to those reported by Morsy *et al.* (2012) and Morsy *et al.* (2015) respectively. Moreover, the study of their ordinary microscopy and SEM detailed morphological characters were compared with previously described forms illustrating some morphometric variations which may be due to their presence in different fish host and environmental conditions.

Juvenile anisakids were previously reported from Egypt in the Red Sea fishes at Hurghada by Abdou and Droden (2007), those of *A. simplex* by Arafa *et al.* (2009) and Morsy *et al.* (2015) and from the Mediterranean Sea by Mohamed and Abdel-Ghany (2011, Morsy *et al.* (2012). They were recorded from the Red Sea at Yemen coast by Al-Zubaidy (2010) and Al-Zubaidy *et al.* (2012**).**

Weerasooriya *et al.* (1986) stressed the high value of the external fine morphology in the identification of larval anisakids reporting that there were clear generic differences between different larval anisakids. The present SEM data agree with those of Morsy *et al.* (2012) which were described from the European seabass marine fishes collected from coasts of Mediterranean Sea, Egypt in the presence of boring tooth and four papillae surround in triangular mouth in anterior extremity, transverse and longitudinal striations on the cuticle and body papillae. However, these authors were concerned with anisakid juveniles; without mentioning the stage of the larvae, which seems to be of Type I as they have a terminal mucron while in the present study type I and type II third stage larvae were identified and differentiated by ordinary microscopy and SEM study. However, the tail with a mucron but papillae-like structures arranged in the tail region did not appear in the present SEM study. Also, Morsy *et al.* (2015) described the SEM surface structures of *Anisakis* sp Type II from the marine greater lizard fish *Saurida undosquamis* captured from water coasts at Hurghada City along the Red Sea in Egypt which were more or less similar to the present study data but from a different fish host.

Anisakids cause serious human disease by eating insufficiently cooked marine fishes containing 3rd stage larvae of different anisakid species (El- Gazzar *et al*, 2004). Infection with type II 3rd stage *Anisakis* larvae was associated with abdominal pain, nausea and diarrhea with very high eosinophilia and the formation of granulomata in gastrointestinal tract if the larvae were not removed (Morsy *et al*, 2017). Freezing for few days and prompt cooking of fish is enough to kill the larvae and prevent infection (Toyoda and Tanaka, 2016).

Studies on prevalence, morphometry, and SEM of not only adults but also juvenile larvae of different anisakids in marine fishes in Egypt is recommended with special reference to type I & type II and their pathological effects on humans.

Conclusion

Anisakis simplex third stage larvae; type I & type II were identified from three Red sea fishes; *Caranx sexfasciatus*, *Cephalopholis miniata* and *Variola louti* brought from Hurghada coasts, Egypt. The prevalence of these larvae was reported and their detailed & SEM ultrastructural morphological characters were described and compared with previously described forms from Red Sea fishes reported from Egypt, Yemen and Arabia Saudi.

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Explanation of plates

Pl. 1: Photomicrographs of larval *Anisakis simplex* (3rd stage larva, type I) showing: A- Whole larva. B-Lateral view of anterior extremity of t larva showing its boring tooth (BT), papillae, excretory pore, nerve ring (NR), esophagus (ES), ventriculus (Vn) and intestine (In). C- High magnification of anterior extremity of larva showing its boring tooth, papillae, excretory pore, nerve ring and esophagus. D- High magnification of posterior extremity of larva showing its anal opening, rectal glands (RG) and mucron (M).

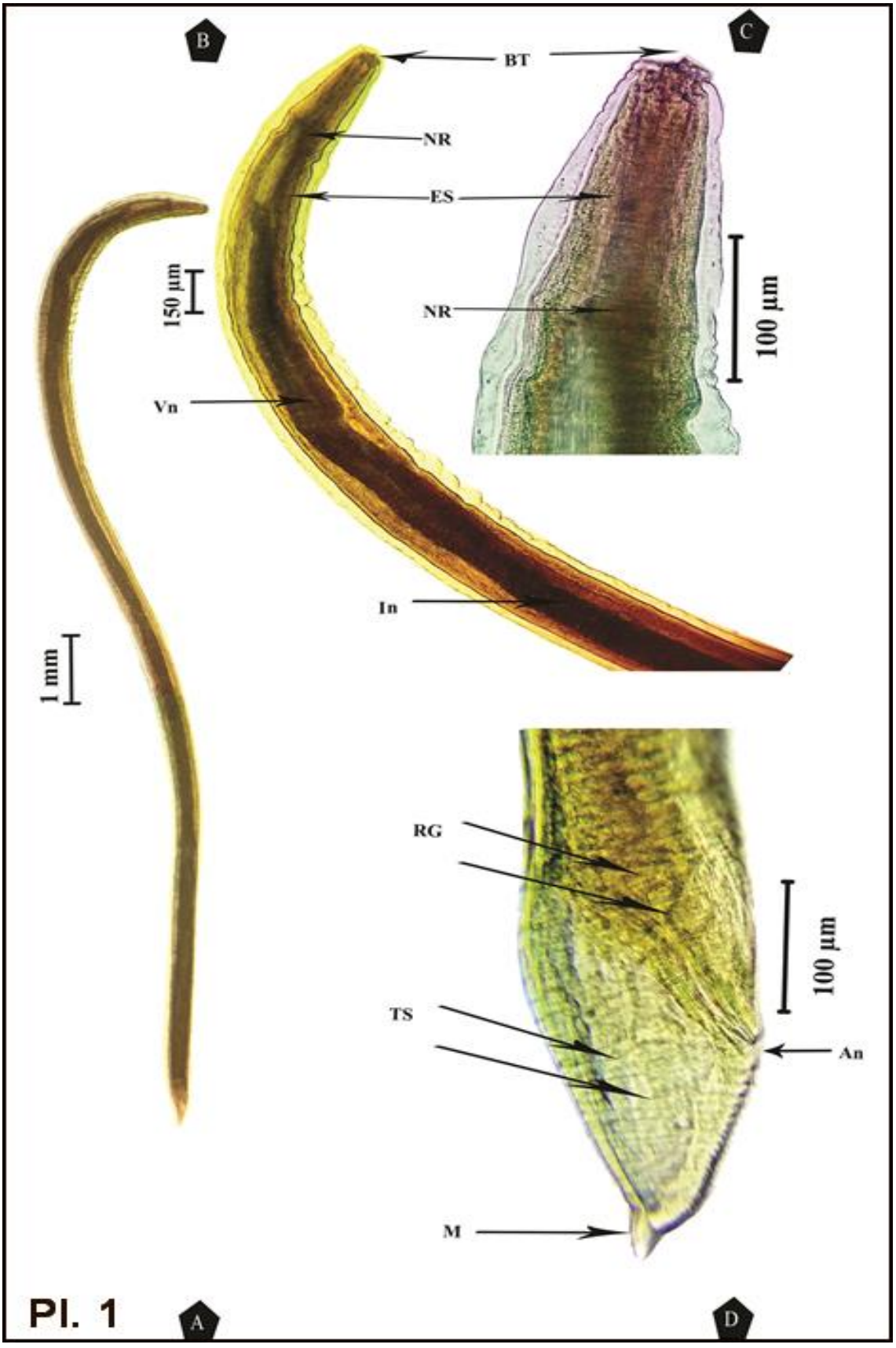
Pl. 2: Line diagram of larval *A. simplex* (3rd stage larva, type I) showing: A-Lateral view of anterior extremity of larva showing its boring tooth (BT), nerve ring (NR), esophagus (ES), ventriculus (Vn) and intestine (In).B- High magnification of the anterior extremity of the larva showing its boring tooth, papillae, nerve ring and esophagus. C- Lateral view of posterior extremity of larva showing rectum (Re), rectal glands (RG), anus (An) transverse striations (TS) and mucron (M).

Pl. 3: SEM micrographs of *A. simplex* (3rd stage larva, type I) showing: A-Anterior extremity of larva showing boring tooth (BT), papillae (Pa) mouth (Mo) excretory pore (EP) and longitudinal annulations (LA). B- Posterior extremity of larva showing longitudinal annulations (LA), transverse striations (TS) and mucron (M).

Pl. 4: Photomicrographs of *A. simplex* (3rd stage larva, type II) showing: A- Whole larva. B- Lateral view of anterior extremity of larva showing its boring tooth papillae, excretory pore, nerve ring, esophagus, ventriculus and intestine. C- High magnification of anterior extremity of larva showing its boring tooth, papillae, excretory pore, nerve ring and esophagus. D- High magnification of posterior extremity of larva showing its anal opening, rectal glands and mucron.

Pl. 5: Line diagram of larval *A. simplex* (3rd stage larva, type II) showing: A-Lateral view of anterior extremity of larva with boring tooth (BT), nerve ring (NR), esophagus (ES), ventriculus (Vn) and intestine (In).B- High magnification of anterior extremity of larva showing its boring tooth, papillae, excretory pore (EP), nerve ring and esophagus. C- Lateral view of posterior extremity of larva showing rectum (Re), rectal glands (RG), anus (An) and tail (Ta).

Pl. 6: SEM micrographs of *Anisakis simplex* (3rd stage larva, type II) showing: A- C. Encapsulated larva coiled in a thin walled cyst. D- Anterior end of encapsulated larva showing prominent boring tooth (BT)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Pl. 1
A
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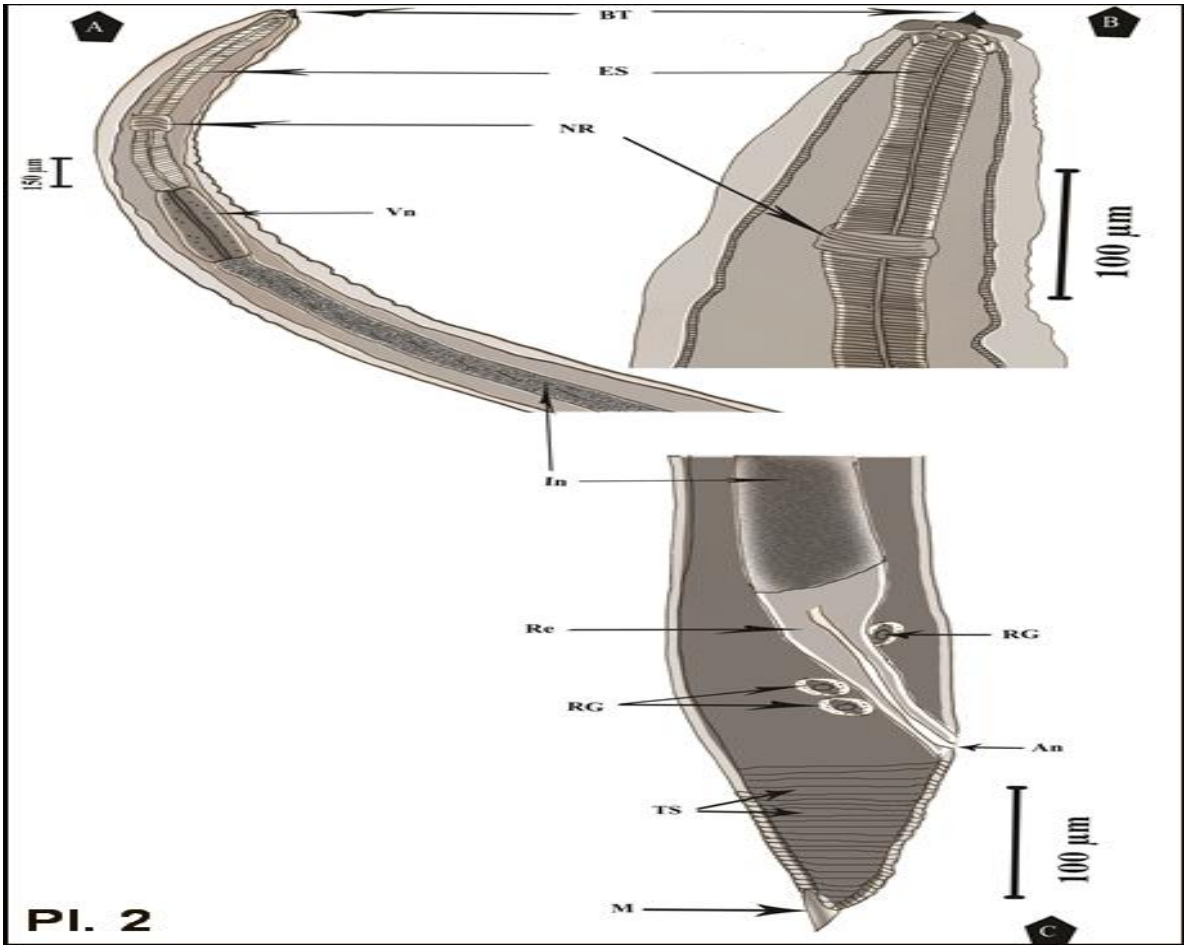
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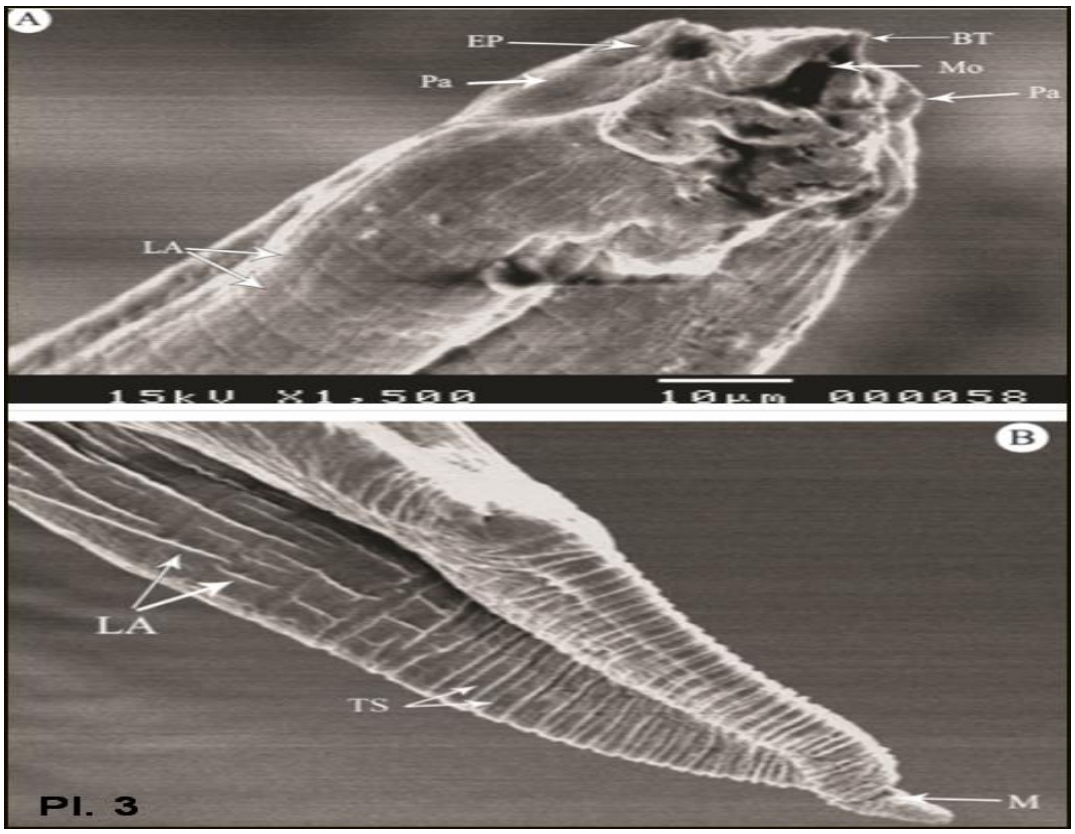
Pl. 2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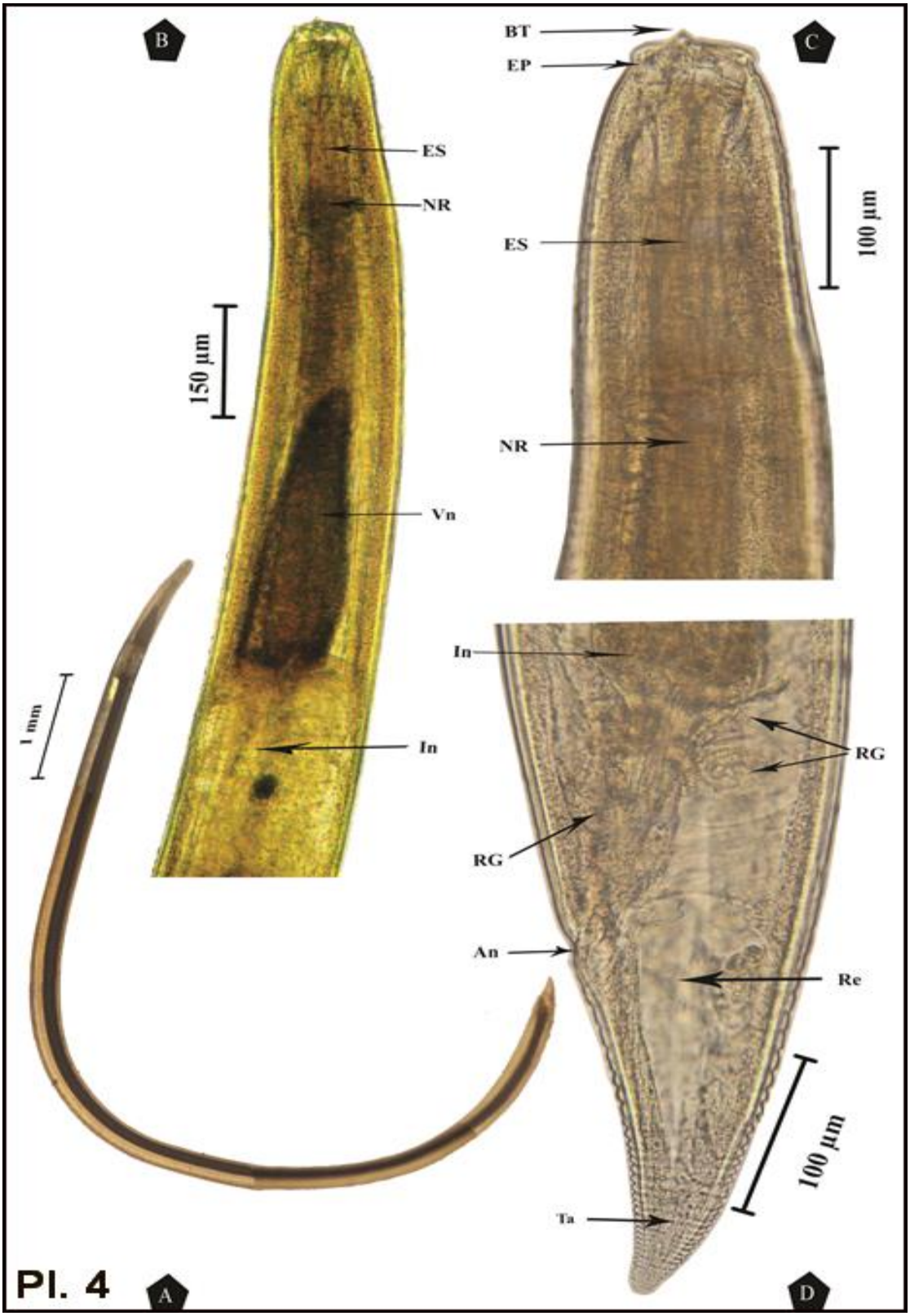
Pl. 3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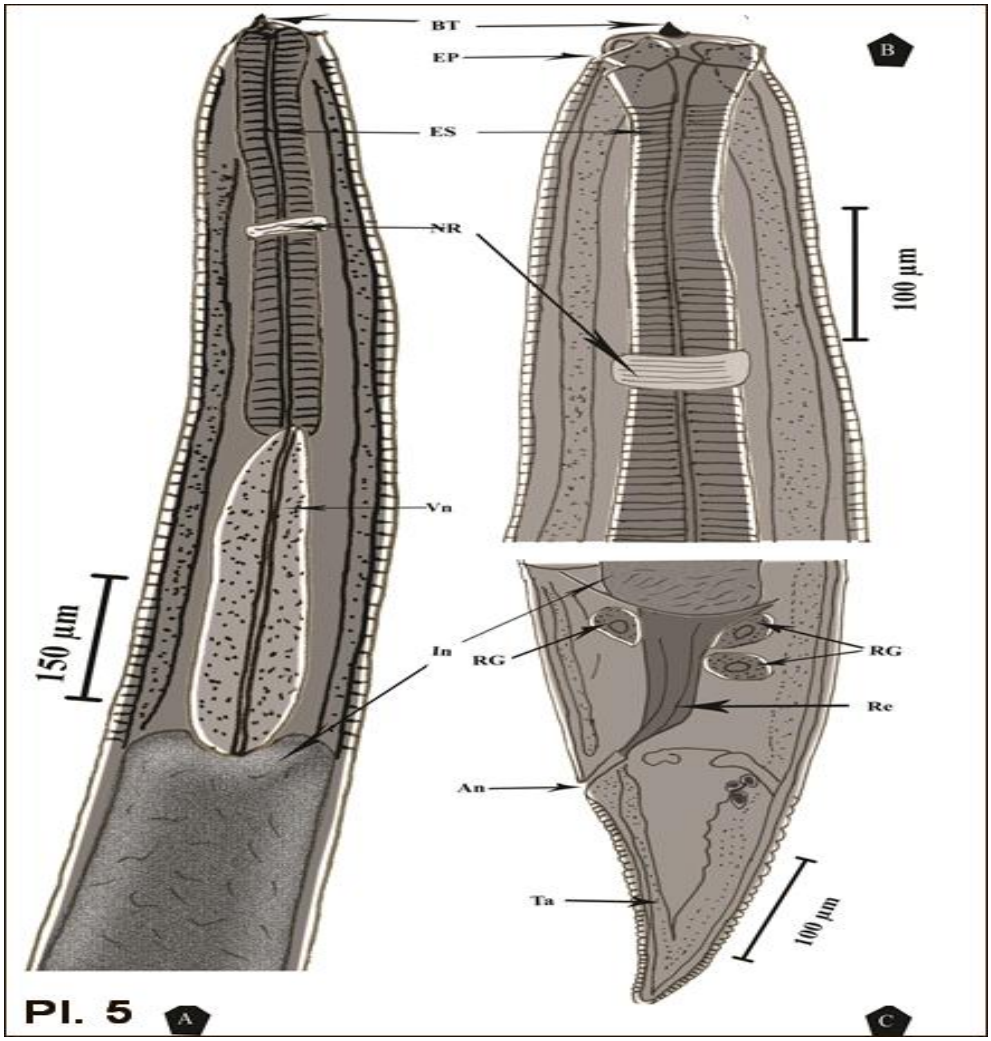
Pl. 4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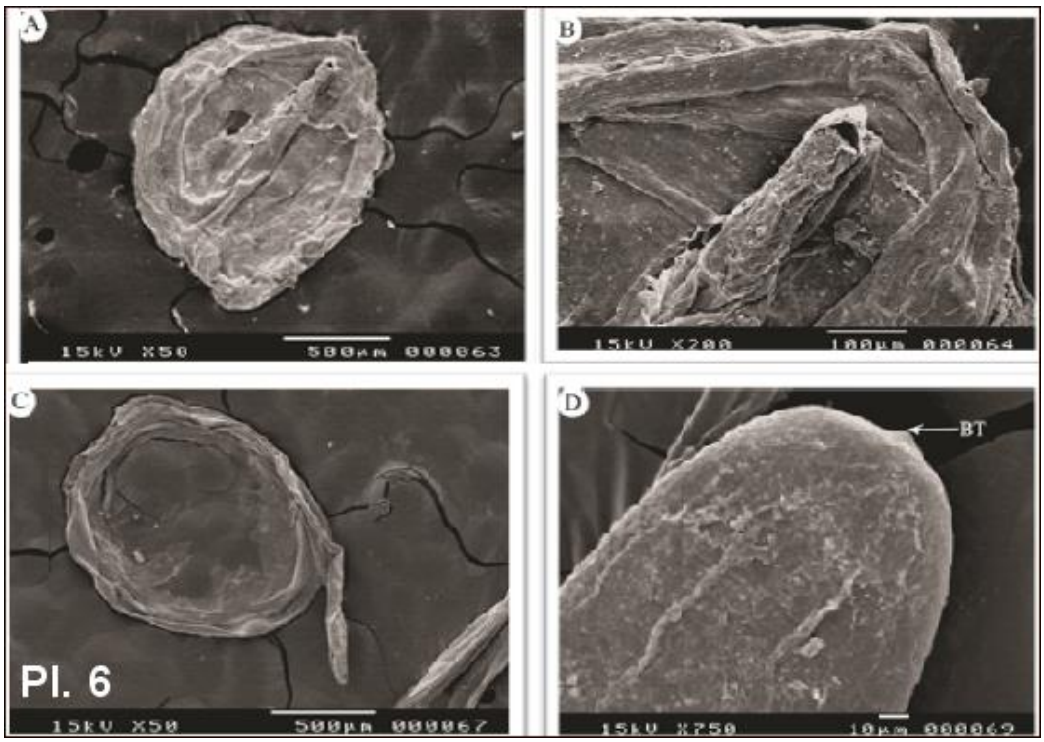
Pl. 5


A
15kV X50 500µm 000063
B
15kV X200 100µm 000064
C
15kV X50 500µm 000067
D
BT
15kV X750 10µm 000069

Pl. 6