Tropical Biomedicine 32(4): 745–752 (2015)

Observations of the rabbit pinworm *Passalurus ambiguus* (Rudolphi, 1819) in domestic rabbits (*Oryctolagus cuniculus*) in Egypt using a scanning electron microscope

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Received 30 March 2015; received in revised form 21 July 2015; accepted 3 August 2015

Abstract. *Passalurus ambiguus* (Rudolphi, 1819) is one of the most common oxyurid nematodes to be found in domestic and wild rabbits worldwide. In order to identify and determine the morphological characteristics of this pinworm, the current research was conducted as a microscopic study, using a scanning electron microscope (SEM). Adult *P. ambiguus* were collected from naturally infected domestic rabbits in Egypt. Identification was made primarily by ordinary microscope, and subsequently, the morphological features were evaluated by SEM. Results indicate that SEM is a powerful tool to identify in detail the morphological characteristics such as the head, male cloacal area, female tail and the female copulatory plugs. All these features confirm the species is *P. ambiguus* and show the effectiveness of the SEM to differentiate *P. ambiguus* from related oxyurid species.

INTRODUCTION

Passalurus ambiguus (Nematoda: Oxyuroidae) is one of the most prevalent gastrointestinal nematodes that infects domestic and wild rabbits, hares, and rodents worldwide (Pritt *et al*., 2012).

Rabbits (*Oryctolagus cuniculus*) are reared for several purposes, including economics, medical research, and as pets (Okerman, 1994). Significant economic losses can accrue from parasitic infections. It has been assumed that *P. ambiguus* is non pathogenic, or is a less pathogenic than others (Okerman, 1994; Hobbs *et al*., 1999; Pritt *et al*., 2012).

The oxyurid nematodes (pinworms) are a large group of nematodes of both medical and veterinary importance (Vicente *et al*., 1997). Identification of the helminth species is a critical point, particularly among pinworms and much depends on ordinary microscopic observations (Pinto *et al*., 2004). Yet few efforts have been made to use the scanning electron microscope (SEM) (e.g. Bin & Chunsheng, 1987) or similar molecular-based tools (Li *et al*., 2014), in the identification of the *Passalurus* species.

The current study aims to examine the *Passalurus* sp. That infects domestic rabbits in Egypt using both the ordinary microscope and the SEM, to identify this species with higher certainty and to determine its characteristic morphology, which may contribute valuable information to knowledge of the oxyurids.

MATERIALS AND METHODS

Study area and samples: Gastrointestinal tracts (GITs) of domestic rabbits were collected from local slaughter-shops in Tanta City, Al-Gharbia Province, in the mid Delta of Egypt. The collected GITs were transferred to the laboratory as quickly as possible.

Parasitological examination: The collected GITs were carefully incised; contents of the large intestines were evacuated into clean Petri dishes, inspected for the presence of adult pinworms by stereomicroscope. All recovered nematodes were washed thoroughly in 0.9% saline to remove host tissue debris, and some were temporarily mounted on glass slides with/without the aid of lactophenol, for morphological identification of the species. The recovered worms were preserved in 10% neutral formalin/70% ethanol for further ordinary microscopic examination. Identification was done using available identification keys (Skinker, 1931; Yamaguti, 1961; Hugot *et al*., 1982; Vicente *et al*., 1997; Petter & Quentin, 2009).

SEM study: Some worms (4 males and 3 females) were prepared for SEM study, as described earlier by Yu & Crites, 1986; Rhee *et al*. 1994. Briefly, worms were fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4ºC and then post-fixed in 2% osmium tetroxide for 4 h. Later samples were dehydrated in graded ethyl alcohol and then dried to critical point in a CPD unit. The processed specimens were mounted over the stubs with double carbon conductivity tape, and a thin layering of gold coat was applied over the samples using an automated sputter coater. These were then scanned under a scanning electron microscope (SEM–model: JOEL-JSM 5300) at the required magnifications, as per the standard procedures of Electron Microscope Unit (EMU), Faculty of Science, Alexandria University, Egypt.

RESULTS

Small whitish worms were collected alive from the ceca and colons of domestic rabbits and these were primarily identified as *Passalurus* sp. Under the stereomicroscope, the female worms were seen to be striated with pointed posterior end and these were comparatively longer than the small posterior coiled males.

Using an ordinary microscope, a more reliable identification could be made of the collected samples. These were identified as *P. ambiguus* and revealed the characteristic shape of the esophagus in both sexes (Figures 1 A & B), with its large distal bulb, and the cervical lateral wings in the female, while these wings were reduced in the male (Figure 1 A). The posterior end of males was markedly coiled, with single short protruded spicule, absence of the gubernuculum, characteristic cloacal ornamentation, and long caudal appendix (Figure 2 A). In contrast the female showed a long tapering straight tail with cuticle ring-like striations (Figure 2 B). Intrauterine eggs were elliptical with one flattened side, double thin-walled, and contained a developing embryo (Figure 2 C).

Using the SEM, the anterior end of *P. ambiguus* was discerned showing the head plate, followed by the typical transverse cuticular striations in both males and females. In females, marked lateral inflations "wings" were seen (Figures 3 A and B). The head of *P. ambiguus* was characterized by 4 head papillae arranged in the dorsal and ventral surfaces and giving a square shape, as well as 2 lateral head amphids. The mouth was triangular in-shape, and edged by 3 teeth-like structures; each one is subdivided longitudinally, giving the appearance of 2 sliding rounding doors. No lips were seen (Figures 4 A and B).

Passalurus ambiguus male genital features were discerned confined in the posterior end, which was typically coiled, with, at the cloacal region, a single, short spicule seen to protrude from the body (Figures 5 A & B), the presence of 2 pairs of large peri-cloacal papillae, the second pair typically sessile, pea-like, while another pair was seen post cloacal, as small and vestigial (Figure 5 B). The male body was seen to continue until it formed a V-shaped divergence (Figure 5 C) and these terminate with a small papillae-like structure (Figure 5 D). Just before this divergence and on its dorsal surface, the caudal appendix begins, but the marked striation is absent. The male caudal appendix was long and pointed.

Females of *P. ambiguus* are characterized by the lateral cuticular wings in the anterior one-third and by the presence of the copulatory plugs on the ventral surface (Figures 6 A and B). In the posterior part of

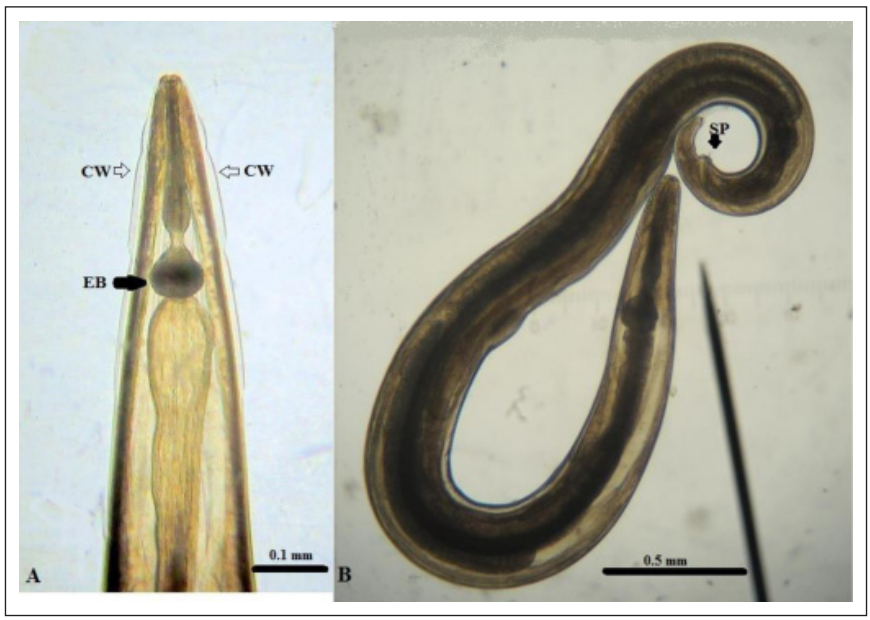

Figure 1. **A.** Anterior end of adult *Passalurus ambiguus* female shows the lateral cervical wings (CW, white arrows) and the characteristic shape of esophagus with its large posterior bulb (EB, black large arrow).
B. Whole male of *P. ambiguus* shows the same esophageal character besides the marked coiled posterior end with short protruded spicule (SP, small black arrow).

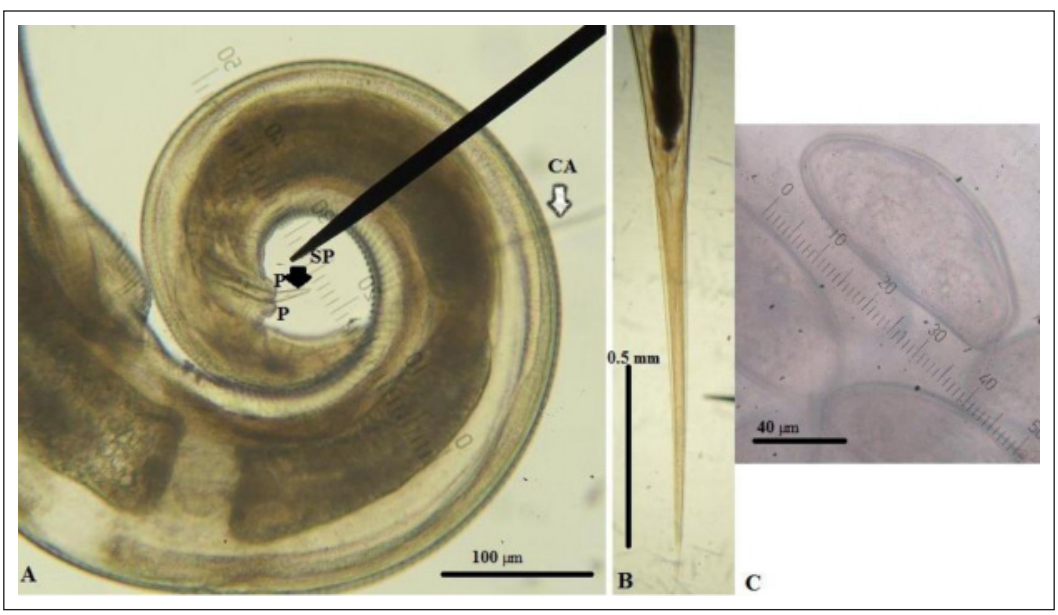

Figure 2. **A.** Magnified posterior end of adult *Passalurus ambiguus* male on lateral view shows the marked coiled posterior end with short protruded spicule (Sp, black arrow), pericloacal papillae (P) and long caudal appendix (CA, white arrow).
B. Posterior end of *P. ambiguus* old female posterior end shows the very long tapering tail.
C. Intrauterine eggs (right) shows the remarkable shape as being double shell, flatten on one side and contains well-developed embryo.

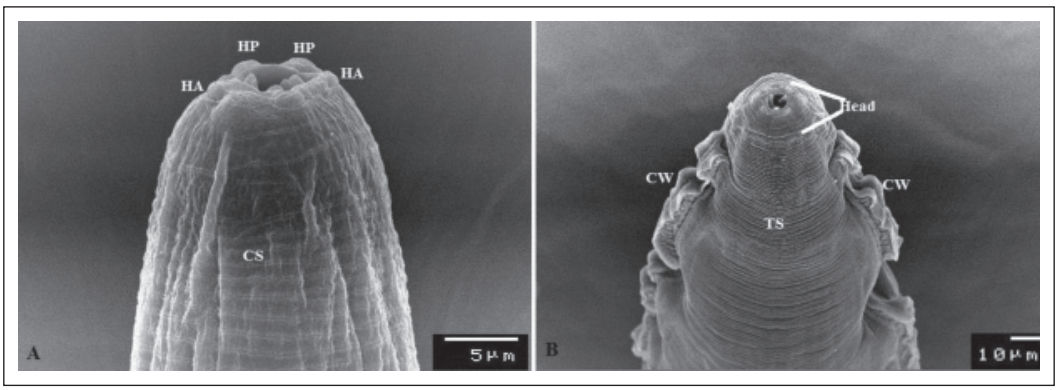

Figure 3. **A.** SEM photomicrographs of anterior end of adult *Passalurus ambiguus* male on lateral view show the marked head papillae (HP), head amphids (HA) and cuticular striation (CS) without neither cephalic nor cervical alae nor wings.
B. SEM photomicrograph of anterior end of *P. ambiguus* old female anterior end (right) ventral view shows the transverse striation (TS) and the lateral cervical wings (CW).

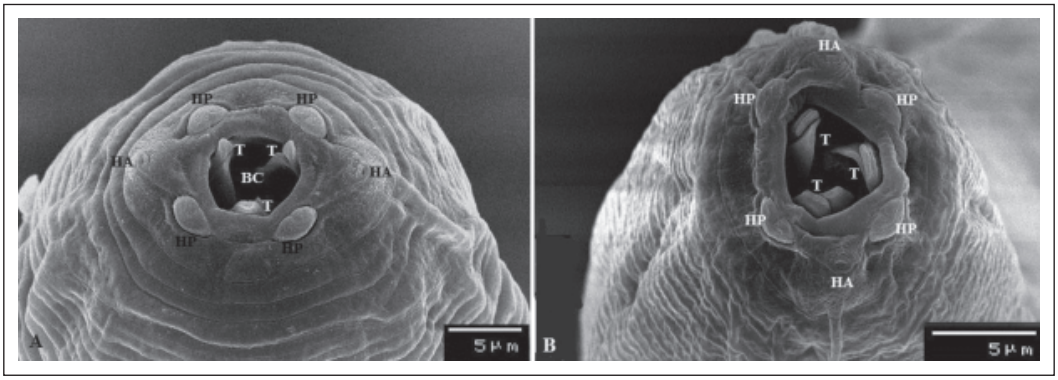

Figure 4. **A. & B.** SEM photomicrographs show the apical view "En-face" of adult *Passalurus ambiguus* (ventral view on left hand and lateral view on right hand) shows the marked 4 head papillae (HP) arranged in a square-like, the 2 lateral amphids (HA) and the large triangular buccal cavity (BC) with three sub-divided teeth (T).

the body; the anal opening was observed in the ventral side as a transverse slit with a dome-like appearance and is characterized by the absence of cuticular striation at the anal region. The tail was long, with marked rings that have a "moniliform appearance" as a result of successive narrow transverse cuticular striation (Figure 7 A), and this tail ends with a very pointed pin-like bare tip (Figure 7 C).

DISCUSSION

Although, *P. ambiguus* is found world-wide in rabbits, hares and rodents, there are few studies that deal specifically with this nematode. In Egypt, *P. ambiguus* is one the most prevalent helminthes found in domestic rabbits, up to 40% of samples are infected with it and younger animals are more commonly infected than adults (Elhawaray, 2009; Ashmawy *et al.*, 2010).

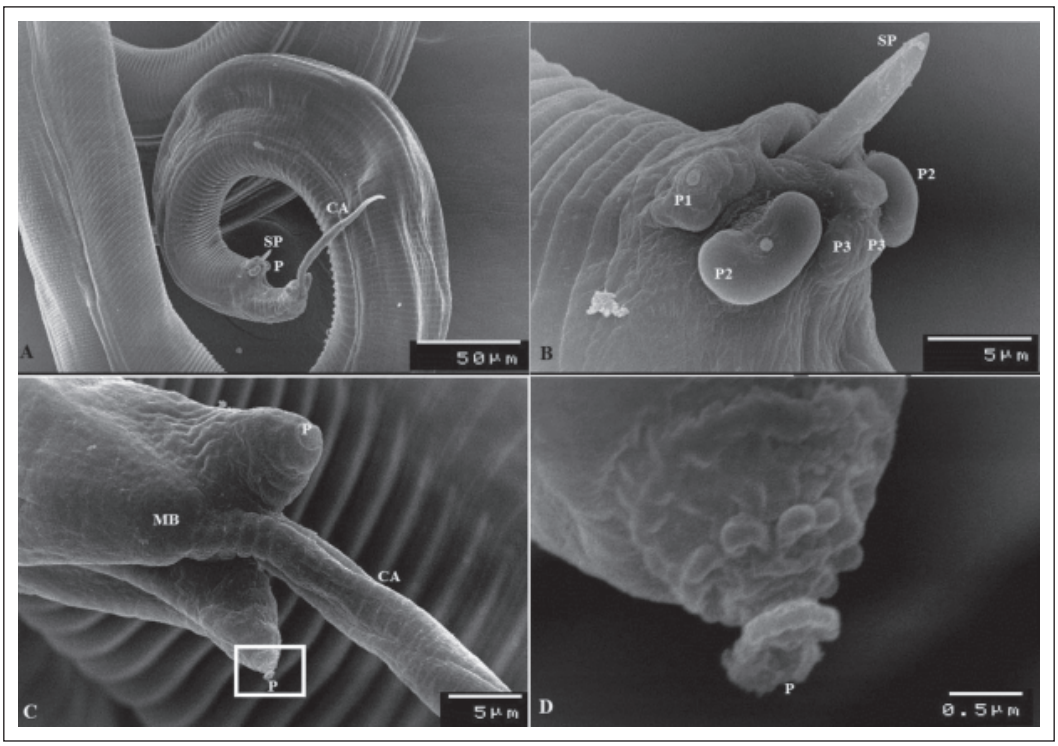

Figure 5. SEM photomicrographs show adult *Passalurus ambiguus* male characters.
A. The marked coiled posterior end shows the transverse cuticular striation; the spicule (SP) protruded out from the cloacal area which surrounded with a 3 pairs of papillae (P); and a very long caudal appendix (CA).
B. A magnified cloacal area shows the short single spicule (SP) protruded out and the presences of 3 pairs of large peri-cloacal papillae (P), the second pair (P2) is typically pedunclated pea-shaped, while the last pair (P3) is sessile and post-cloacal.
C. The dorsal view of the origin of the caudal appendix shows the end of the male body (MB) with a V-shaped divergence those end with a small papillae-like (P) which appears in white square and magnified in (**D.**), just before this divergent and on its dorsal surface the caudal appendix begin (CA).

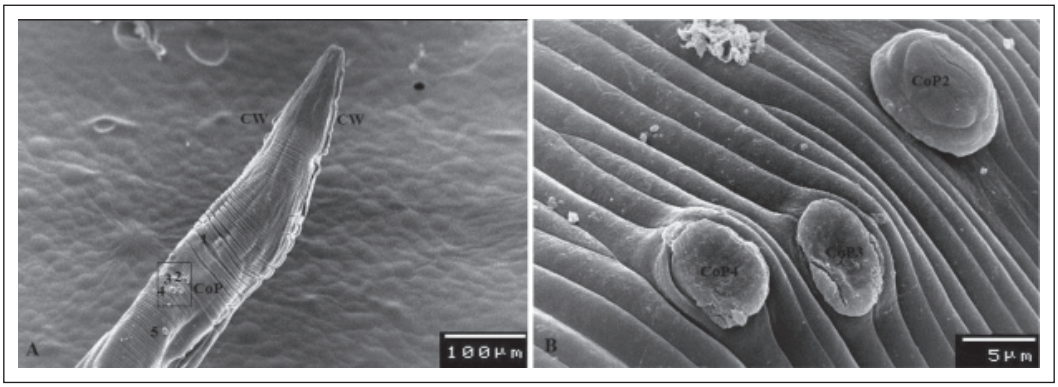

Figure 6. SEM photomicrographs show adult *Passalurus ambiguus* old female anterior end on the ventral view.
A. Shows the transverse cuticular striation with presence of the cervical or lateral wings (CW) and the 5 copulatory plugs (CoP 1-5) at the anterior one-third of the body.
B. Magnified copulatory plugs (CoP2, 3 and 4).

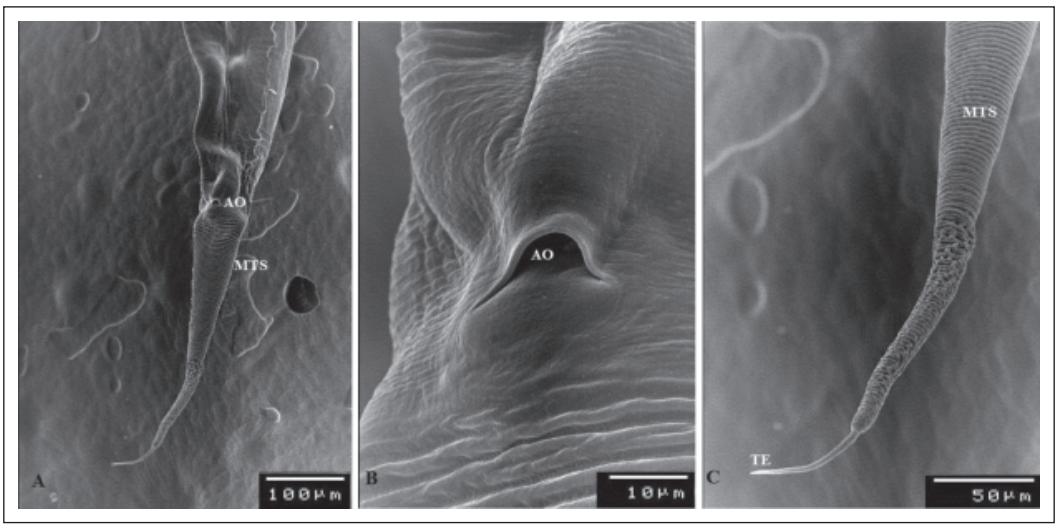

Figure 7. SEM photomicrographs show adult *Passalurus ambiguus* old female posterior end on the ventral view.
A. On the left shows the female tail characters; the anal opening (AO), the origin of tail behind it; the marked moniliform transverse cuticular striation (MTS) of the tail.
B. Magnified dome like anal opening (AO) with absence of striation at the anal region.
C. The very pointed pin-like bare tail end (TE).

The results seen under the ordinary microscope (Figures 1 & 2)with regards to the morphological features of adults and eggs of *P. ambiguus* are in close agreement with those reported in other studies (Skinker, 1931; Hugot *et al*., 1982; Pinto *et al*., 2004). The most important features are the characteristic shape of the esophagus, the male genital area and the female posterior end. Nevertheless, the literature advocates morphological differentiation between two closely-related pinworms, namely *"P. ambiguus"* and *"P. nonanulatus*".

The main differences in morphological features are that the *P. nonanulatus* female tail lacks the characterstic moniliform appearance and the number and position of cloacal papillae found in males (Skinker, 1931; Hugot *et al*., 1982). It may be difficult to distinguish between these features, using an ordinary microscope, (see Hugot *et al*., 1982). It was seen that the SEM showed the advantages of high-resolution for discerning the ultra structure of the nematode species, particularly the oxyurids. Using the SEM might improve morphological identification and also better understanding of taxonomic and phylogenetic studies (Eisenbach, 1986; Yu & Crites, 1986).

Our SEM micrographs of adult pinworms provide conclusive evidence that the species that was collected for study is *P. ambiguus* and we contribute some observations on its morphology. Briefly, the anterior ends of the female are marked with lateral cuticle wings-like infilations which appear just behind the head and continue in the anterior third of the body (Figures 1 A & 3 B); en-face vision demonstrated head features that include two lateral amphids and the square like head papillae, the absence of lips, and the triangular mouth opening which has dorsally and ventrally three subdivided dents or teeth-like structures (Figures 4 A & B). These important morphological features, which are key for the identification and the differentiation of genus *Passalurus* from other genera of the family Oxyuridae (Yamaguti, 1961; Hugot *et al*., 1982; Vicente *et al*., 1997; Petter & Quentin, 2009), are difficult to see by ordinary microscopical examination. The characteristics seen by SEM also are helpful for the differentiation of *"P. ambiguus"* from *"P. nonanulatus*". The findings on the head

of *P. ambiguus* agree with those of Hugot *et al.*, 1982; Bin & Chunsheng, 1987. Phasmids are important characteristics of the class Secernentea and were seen clearly in both males and females (Figures 4 A and B), with SEM.

Interestingly, Bin & Chunsheng, 1987 in their short note described the presence of lips in *P. ambiguus*, although nearly all available keys (Hugot *et al.*, 1982; Vicente *et al.*, 1997; Petter & Quentin, 2009) and our works do not mention such structure. The erroneous reference to such a morphological feature may be attributed to the fact that they considered the head papillae or the 3 teeth-like structures to be lips. It is well-known that where lips appear they are located outside the buccal cavity, to guard it; being able to determine the presence or absence of lips and pharyngeal teeth in Oxyuridea is of great morphological value (Petter & Quentin, 2009). In the specimens we examined, no outer lips were discerned, while 3 subdivided pharyngeal teeth-like structures were seen inside the triangular mouth (Figure 4 B).

Using SEM, the cloacal area topography in males was clearly observed. Estimation of the number and position of papillae in this area varies from one author to another, as Skinker observed, 1931 and as confirmed by the observations of Hugot *et al.*, 1982, features used by later researchers to differentiate *P. ambiguous* from *P. nonanulatus*. In our examined specimens, 3 pairs of papillae were observed (Figure 5 B). The first 2 pairs were peri-cloacal and larger than the last pair, which was small, sessile and positioned just post-cloacal. This finding is in agreement with the description of male *P. ambiguous* given by Hugot *et al.*, 1982. The nature of these papillae may be attributed to the nature of the insemination process in *Passalurus*. Another pair of small papillae are situated at the point where the tail narrows and the caudal appendix arises. This is also a feature of *P. ambiguus* males, according to Skinker, 1931. The pinworm males in the present study therefore are *P. ambiguus*.

The SEM was superior to the ordinary microscope in demonstrating another important feature, "the copulatory plugs", that are unique to the female *Passalurus* species (Figures 6 A and B). It is known that *P. ambiguus* has an exceptional route of insemination called the "traumatic insemination", in which method the male incises the intact cuticle of the female and inseminates, and the cuticle refolds and closes, forming the characteristic plugs (Hugot *et al.*, 1982). The position of the copulatory plugs in the current study is in close agreement with that described by Hugot *et al.*, 1982, but while they detect 9 plugs, we detect only 5. The variation in the number of plugs number is not a critical point; it can be explained by the difference in mating nature.

The adult female tail topography was a point of differentiation between *P. ambiguus* and *P. nonanulatus*; as the latter species lacks the typical transverse cuticular striations known as the moniliform appearance, that are found in *P. ambiguus*. This characteristic appearance can be detected by the ordinary microscope, but it is best seen by the SEM (Figure 7). The results here indicate that the collected samples of females are those of *P. ambiguus*.

In conclusion, the results in the current study indicate that based on the unique morphological features that were observed by SEM, the pinworm species infecting domestic rabbits in Egypt is conclusively identified as *P. ambiguus*.

Acknowledgments. The authors wish to express their thanks to veterinarian Nuha Amaar for her help during collection of the study specimens. They are also grateful to staff members at EMU, Faculty of Science, Alexandria University for their sincere cooperation during the study. The authors appreciate the remarks and suggestions of Professors M.A. El-Seify and A.Y. Desouky, which improved the work. The authors also appreciate the efforts of Agatha Bardoel for language revision and the anonymous reviewers for their remarks that enriched the manuscript.

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