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Morphological and Molecular Identification of Some Intestinal Helminths Infesting the Domestic Pigeon (*Columba livia domestica*) at Ismailia, Egypt

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ABSTRACT

The study identified helminths infecting the domestic pigeon, *Columba livia domestica* using conventional methods (Light and Environmental scanning electron microscopes) as well as the newly introduced molecular biology methods. 120 pigeons were purchased from Ismailia, Egypt during the period from December, 2015 to November, 2016. Light and Environmental scanning electron microscopes were used to study the general morphology and the surface features of the recovered parasites. The identification of the recovered helminths was confirmed using molecular biology techniques. Four helminths were recorded; one nematode (*Ascaridia columbae*) and three cestodes (*Cotugnia polyacantha, Raillietina beveridgei* and *Raillietina echinobothrida*). The total helminths prevalence was 58.3%. Parasites DNA sequence data was used in Blast test, in order to confirm the identities of recovered cestodes. The phylogenetic tree of *C. polyacantha* picked only one sequence belong to *Cotugnia sp.* from India, in turn, this may be the first time for *C. polyacantha* 18S rRNA to be submitted to GenBank from Egypt. Also, PCR reaction positively identified our nematode as *A. columbae.* Therefore, it is recommended to use molecular technique in helminths identification as the main methodology for correct identification especially in closely related species.

Keywords: *Columba livia domestica*, ESEM, Helminths, Molecular biology, Morphology.

INTRODUCTION

The poultry industry has been confronted with various parasitic diseases of economic significance (Anwar *et al.,* 2000). Pigeons have shown high prevalence of gastrointestinal helminths and protozoan infections (Ghazi *et al.,* 2002). Pigeons are cosmopolitan birds (Sari *et al.,* 2008) and those of the order: Columbiformes can be found, virtually, in every town and city around the globe (Marques *et al.,* 2007). Among which is the domestic pigeon *Columba livia domestica* that rose for food production (meat and eggs). Parasitism is gradually being accepted as one of the major selective forces affecting avian life histories. Birds can be parasitized by a wide variety of ecto and endoparasites (Marques *et al.,* 2007 and Sivajothi and Reddy, 2014).

The common internal parasitic infections occur in poultry include cestodes and nematodes that cause considerable damage and great economic losses to the poultry industry. Furthermore, helminths can make the flock less resistant to diseases and exacerbate existing disease conditions (Katoch *et al.*, 2012).

Nematodes are considered to be most important group of helminth parasites of poultry both in number of species and the degree of damage they cause; the main genera include *Ascaridia, Heterakis, Syngamus* and *Capillaria* (Matur and Dawam, 2010). Some species of *Ascaridia* have been found in pigeons (Foronda *et al.,* 2004). *A. columbae* is a common parasite found in the small inte-stine of pigeons. *Raillietina* species are considered one from the most important cestodes of poultry. They are common in tropics, where the poor standard husba-ndry practices and climatic conditions are favorable for the development of the parasites (Tadelle and Ogle, 2001). Keeping in view the severity of the parasitic helminths, our research plan aims to identify the intestinal helminths infecting the domestic pigeon (*Columba livia domestica*) in Ismailia governorate using light and scanning electron microscopy coupled with molecular identification

MATERIALS AND METHODS

The Birds collection:

120 domestic pigeon (*Columba livia domestica*) were purchased from a birds market in Ismailia province, Egypt during the period from December, 2015 to November, 2016. They were immediately transported to the laboratory and were killed by anesthesia using overdose of chloroform.

Helminths identification:

Pigeons were dissected according to the method described by Al-Hussaini and Demian (1982) and the ileum was taken out separately in Petri-dish containing warm saline solution (0.9%) (37°C). Dissected organ were carefully examined under Wild M3Z Continues Zoom Stereoscope for presence of parasites. Collected parasites from infected pigeons were washed several times in warm saline solution to remove mucous and other host debris and then sorted into nematodes and cestodes.

Specimens were preserved and cleared with Lactophenol. Glycerol jelly method used for nematodes specimens after Fleck and Moody (1993). The parasites were identified under binocular microscope light microscope according to Soulsby (1982) and Yamaguti (1961). The measurements of helminths were made with a calibrated ocular micrometer. Large nematode specimens were measured directly for their total length prior to mount. The parasites were examined and photographed by using Axiostar Plus (Carl Zeiss, Gottingen, Germany) microscope equipped with Canon (Pc 1200 power shoot

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A641) digital camera using Zoom Browser Ex software at the central lab of Zoology Department, Faculty of Science, Suez canal university, Ismailia.

Environmental scanning electron microscopy (ESEM)

Samples were preserved in 70% ethanol; their morphological ultrastructure features were studied using Environmental Scanning Electron Microscope (Inspect S; FEI, Holland) at Electron Microscopy Unit of Theodor Bilharz Research Institute (TBRI) using standard method.

Molecular identification

Identification of the isolated parasites was confirmed by DNA barcoding technique. Parasites were kept frozen at (-18°C) till the time of DNA extraction. Concentrations of genomic DNA (ng/µl) and purity (260/280 nm) were determined using Nanodrop (1000) spectrophotometer after calibration with sterilized double distilled water as a blank. Agrose gel electrophoresis was used to detect the quality of DNA. The genes 18S rRNA and ITS2 for cestodes and mitochondrial genome (mt) for *A. columbae* were amplified with the universal primers described in table (1). PCR amplification was carried out in T3 thermocycler (Biometra) using the following thermal conditions: 5 min at 95°C of the initial denaturation step and 30 sec at 95°C of the second denaturation step for both *Ascaridia columbae* and flat worms followed by annealing step (50°C for 30 sec, *A. columbae*; 55°C for 45 sec, flat worms), then the extension step was 72°C for 30 sec at for *A. columbae* and 72°C for 45 sec, for flat worms. The reaction was terminated by a final extension step (7 min at 72°C and finally kept at 4°C). Molecular analysis of the obtained DNA sequence data was carried using the software publically available on the web (https://blast.ncbi.nlm.nih. gov/Blast.cgi).

Table (1): The primers sequences used in 18S rRNA, ITS2 genes and mt genome amplification.

Target agent	Primer name	Primers sequences	Reference
Ascaridia columbae **mt**	ACC2C1 Forward	5' TAGTATGTGATGTTTGGGAATGCTT 3'	Liu *et al*., 2013
	ACC3C2 Reverse	5' ATAGAAGGCACAGCCCAAGAATGAA 3'	
Raillietina sp. Flatworm **ITS2**	3S Forward	5'GGTACCGGTGGATCACTCGGCTCGTG 3'	Ramnath *et al*., 2014
	A28 Reverse	5'GGGATCCTGGTTAGTTTCTTTTCCTCCGC3'	
Cotugnia sp. **Flatworm 18S rRNA**	18S Forward	5' TTAAGCCATGCATGTCTAAG 3'	Ghobashy and Taeleb, 2015
	18S Reverse	5' GACTACGACGGTATCTAATC 3'	

RESULTS

Recovered helminths

Four helminths species are recorded from the pigeon's ileum part. They are one nematode (*Ascaridia columbae*) and three cestodes (*Cotugnia polyacantha*, *Raillietina beveridgei* and *Raillietina echinobothrida*). The overall prevalence of infection was 58.3%. *A. columbae* body was provided by transverse striations. There are 2 narrow lateral alae or flanges extending along the anterior half of the body. The mouth is surrounded by 3 globular, trilobed, equal seize lips with each lip has 2 cervical papillae. The oesophagus is club shaped without posterior bulb. Male measures 26-28 mm long and 0.73-1 mm wide. The oesophagus measures 1.8-2.3 mm long. The precloacal sucker is provided with strong chitinized wall and it measures 0.19-0.21 mm in diameter. The spicules are strong and equal or slightly subequal with 1.4-1.6 mm long. There are 13 pairs of caudal papillae. Female measures 37.9-40.3 mm long and 0.9-1.1 mm wide. The oesophagus measures 2.1-2.4 mm long. The tail is long, narrow and pointed. The vulva aperture is nearly posterior to the middle of the body (Figures 1 and 2). Scolex of *C. polyacantha* measures 0.3-0.4 X 0.2-0.3 mm diameter with four unarmed circular suckers, each measures 0.09-0.1 mm in diameter. Large retractile and oval rostellum measures 0.1-0.2mm in diameter. It armed with hooks that arranged in 2 rows. Behind these hooks, numerous very flat scaly spin shaped forming indentation at the base of rostellum. Mature segment contains two set of reproductive organs and measures 0.6-0.8 X 2.5-2.8 mm with 2 genital pores. Genital pores located marginally at the middle of the segment sides one on each side. Gravid segment measures 1.2-2 X 4-5 mm; it filled with egg capsules, each with single egg. The testes are small in size, rounded in shape, 90 to 100 in numbers, lying in a single field, in posterior half of the proglottids (Figures 3 and 4).

Concerning *Raillietina* species, both *R. beveridgei* and *R. echinobothrida* have scolex (0.4-0.7 X 0.2-0.5 mm; 0.2-0.3 X 0.3-0.5 mm) with four armed circular suckers (0.13-0.2 mm; 0.1-0.3 mm). They have large retractile and oval rostellum (0.1-0.3 mm; 0.1-0.2 mm) armed with hooks that arranged in 2 rows. Mature segment (1.2-1.6 X 0.3-0.4 mm; 0.42-0.45 X 0.6-0.8 mm) with unilateral, single genital pores. Gravid segment (0.3-0.5 X 2.3-2.6 mm; 0.3-0.5 X 0.4-0.7 mm) filled with egg capsules, each with more than one egg, respectively. The testes are small in size, rounded in shape 5-9 in numbers in *R. beveridgei* and 20-25 in *R.*

echinobothrida (Fig. 5). Body proglottids of *R. beveridgei* have a smooth surface as they appear in the ESEM micrographs (Fig. 6) and are with longitudinal folds in *R. echinobothrida* (Fig. 7).

PCR amplifications and analysis of 18S rRNA and ITS2 genes for the cestode samples and mt genome for *A. columbae*

Nematode samples (*A. columbae*) are analyzed and they gave higher purity (standard: 1.82) and low concentration of DNA 93 ng/µl while the 3 samples of cestodes species (*R. beveridgei, R. echinobothrida, C. polyacantha*) gave purity (1.8-1.79-1.82) and gave low concentration (75-102-88 ng/µl) respectively. The nematodes and cestodes specimens were identified according to the morphological criteria; as *A. columbae* for nematode and *C. polyacantha, R. echinobothrida and R. beveridgei* for cestodes, then they were molecular identified, *C. polyacantha* were identified based on 18S rRNA gene and the *Raillietina* species were identified based on ITS2 and *A. columbae* were also identified based on mt genome. The PCR product molecular size was 178 bp for *A. columbae*, 800 bp for *C. polyacantha*, 450 bp for *R. echinobothrida* and 1000 bp for *R. beveridgei* (Fig. 8).

18S rRNA and ITS2 sequencing, editing and submission to GenBank

The three sequences of *C. polyacantha, R. beveridgei and R. echinobothrida* had been blasted on Genbank and the result for each one as following: *C. polyacantha* gave maximum identity 99%, 0.0 E value and query cover of 100% with *C. polyacantha* (KR082007.1) from India. While *R. beveridgei* gave maximum identity 99%, 0.0 E values and query cover of 100% with *R. beveridgei* (AY382318.1) from Australia and *R. echinobothrida* gave maximum identity 99%, 0.0 E value and query cover of 100% with *R. echinobothrida* (JN797628.1) from India. Editing nucleotide sequences for 18S rRNA for *C. polyacantha* and ITS2 for 2 species of *Raillietina* in the current study are shown in table (2) with their accession number on GenBank.

Phylogenetic analysis

The phylogenetic tree *R. beveridgei and R. echinobothrida* using the obtained ITS2 DNA sequence data as well as the published sequences of related cestodes is shown in figure (9). *C. polyacantha* phylogenetic tree was constructed using our partial 18S rRNA sequence and the related sequences available on GenBank are shown in figure (10).

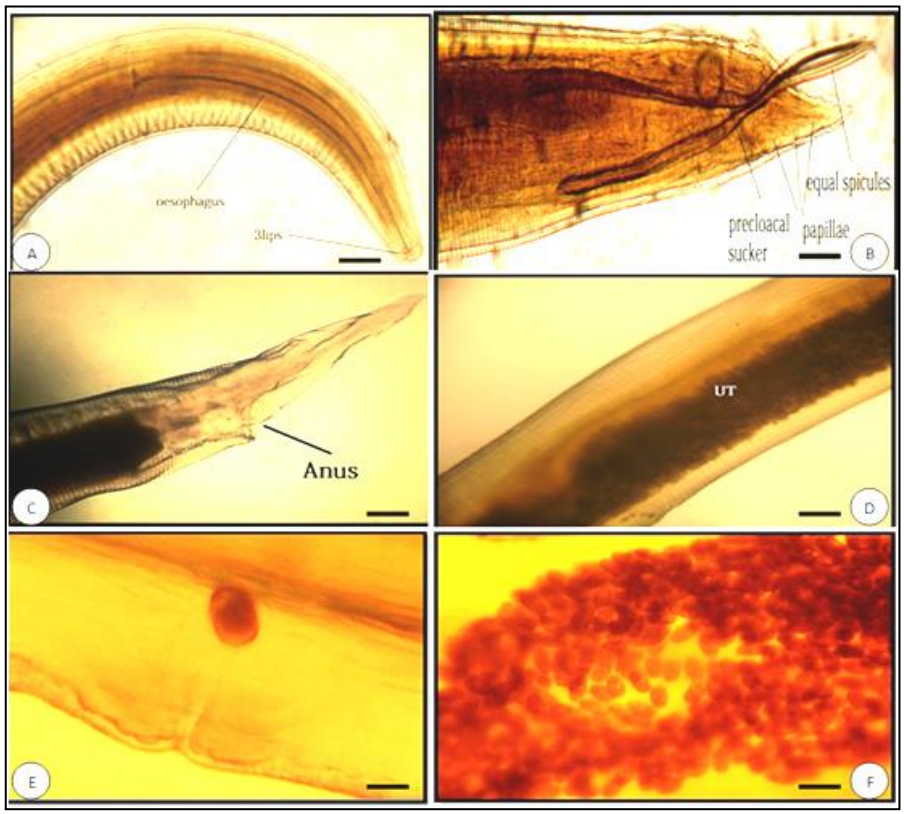

Figure (1):*A. columbae* (A) Anterior end, showing the club-shaped oesophagus and the three lips. Scale bar =100 µm. (B) Posterior end of male showing 2 spicules, precloacal sucker and caudal papillae (Lactophenol). Scale bar =100 µm. (C) Posterior end of female showing the anus and the pointed end. Scale bar =100 µm. (D) The middle part of female showing uterus with eggs (Lactophenol). Scale bar =100 µm. (E) The middle part of female showing the vulval opening (Lactophenol). Scale bar =25 µm. (F) The middle part of female showing the oval-shaped, thick shelled eggs (Lactophenol). Scale bar =25 µm.

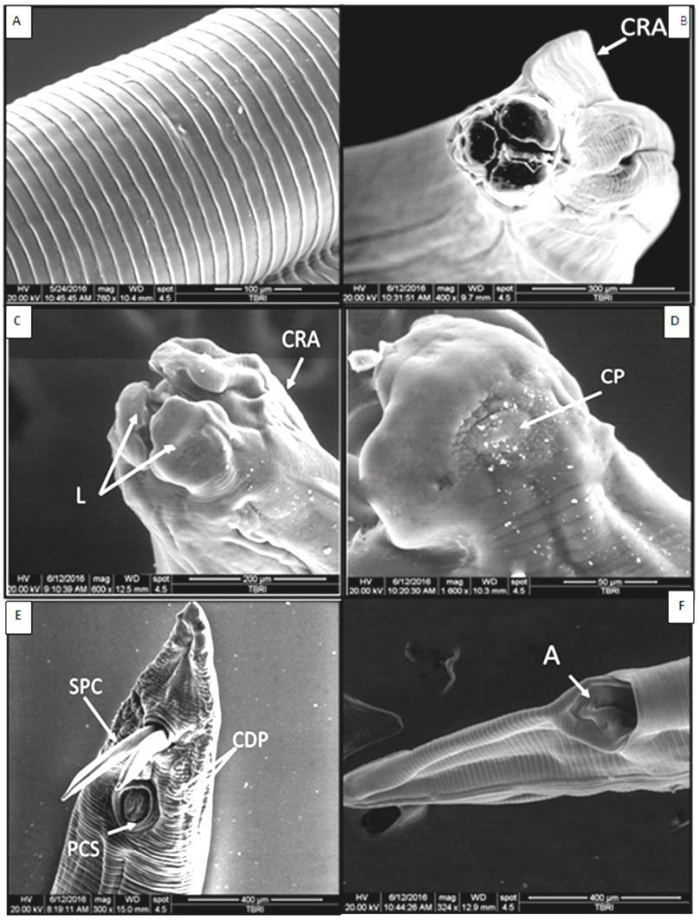

Figure (2): ESEM of *A. columbae* showing (A) cuticular surface of the body; (B) The narrow lateral cervical alae (CRA); (C) The anterior part showing mouth with 3 trilobed lips (L) and cervical alae (CRA); (D) The cervical papillae (CP) of one lip of the mouth; (E) The posterior end of male showing the pre-cloacal sucker (PCS), caudal papillae (CDP) and two spicules (SPC); (F) The posterior end of female showing the anus (A).

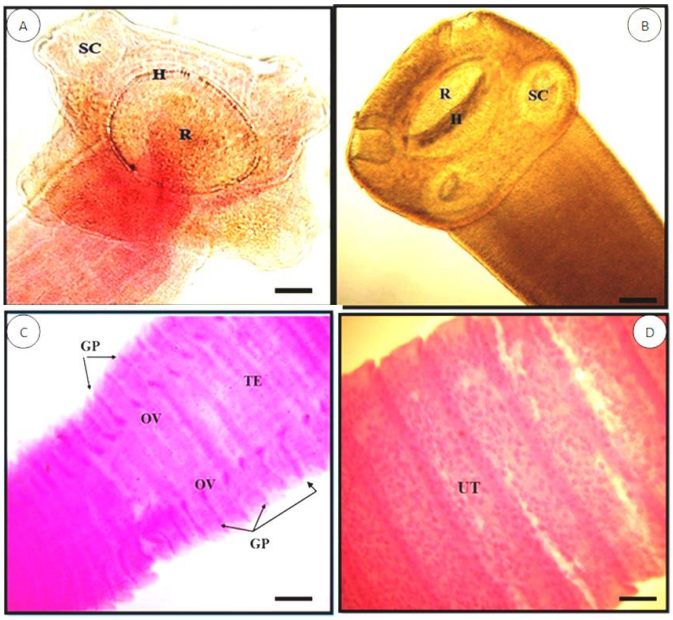

Figure (3): Whole amount of *C. polyacantha;* (A and B) the anterior part showing rostellum (R) with hooks (H) and 4 suckers (SC) (Acetic alum carmine), (Lactophenol). (C) Mature proglottids showing 2 ovaries (OV), several testes (TE) and genital pores (GP) on both sides of each proglottid (Acetic alum carmine). (D) Gravid proglottids showing eggs filling the uterus (Acetic alum carmine). Scale bar =100 µm for all photos.

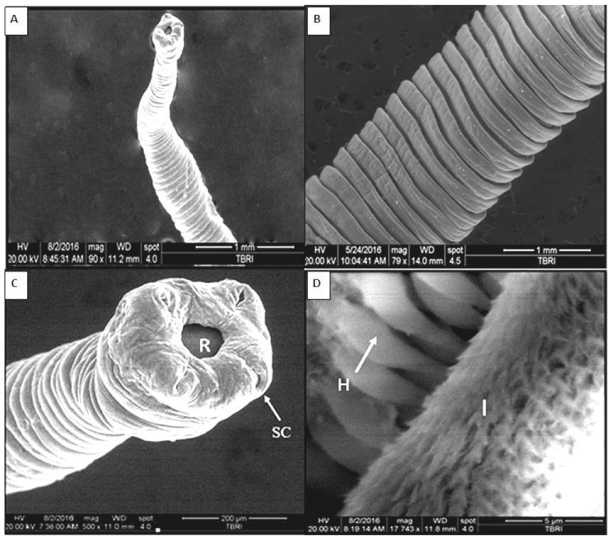

Figure (4): ESEM of *C. polyacantha* showing (A) scolex and strobila with numerous proglottids; (B) Body proglottids. (C) The anterior part showing rostellum (R) and 4 suckers (SC); (D) The anterior part showing hooks (H) and indentation (I).

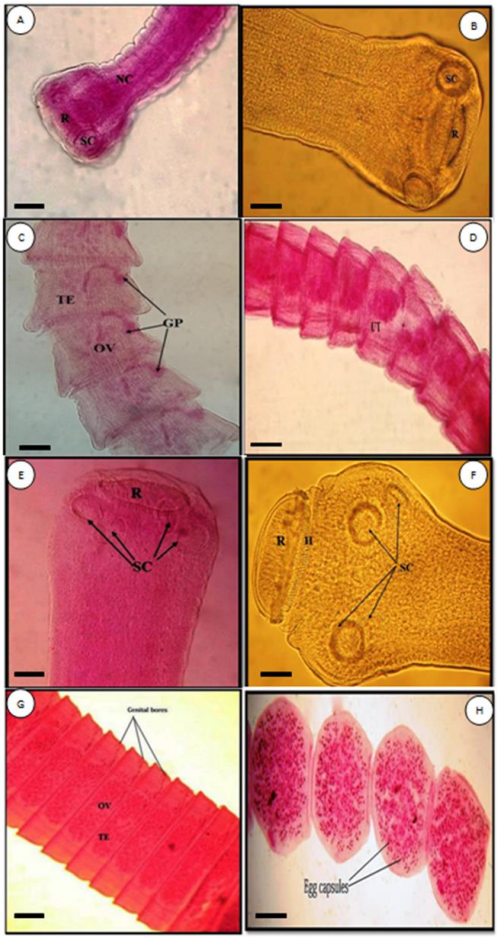

Figure (5): *R. beveridgei*: (A and B) Scolex with rostellum (R), two suckers (SC) and short neck (NC) (Acetic alum carmine), (Lactophenol. A: Scale bar =100 µm. B: Scale bar =50 µm. (C) Mature segments showing ovary (OV), testes (TE) and lateral genital pores (GP) (Acetic alum carmine) Scale bar =100 µm. (D) Gravid segments; uterus with eggs (UT) (Acetic alum carmine) Scale bar =100 µm. *R. echinobothrida* showing (E) Scolex with rostellum (R), four suckers (SC) and short neck (Acetic alum carmine) (Acetic alum carmine). Scale bar =100 µm. (F) Rostellum (R) with two rows of hooks (H) and 4 suckers (SC) (Lactophenol). Scale bar =100 µm. (G) Mature segments showing ovary (OV), testes (TE) and lateral genital pores (GP) (Acetic alum carmine) Scale bar =100 µm. (H) Gravid segments showing egg capsules (Acetic alum carmine). Scale bar =100 µm.

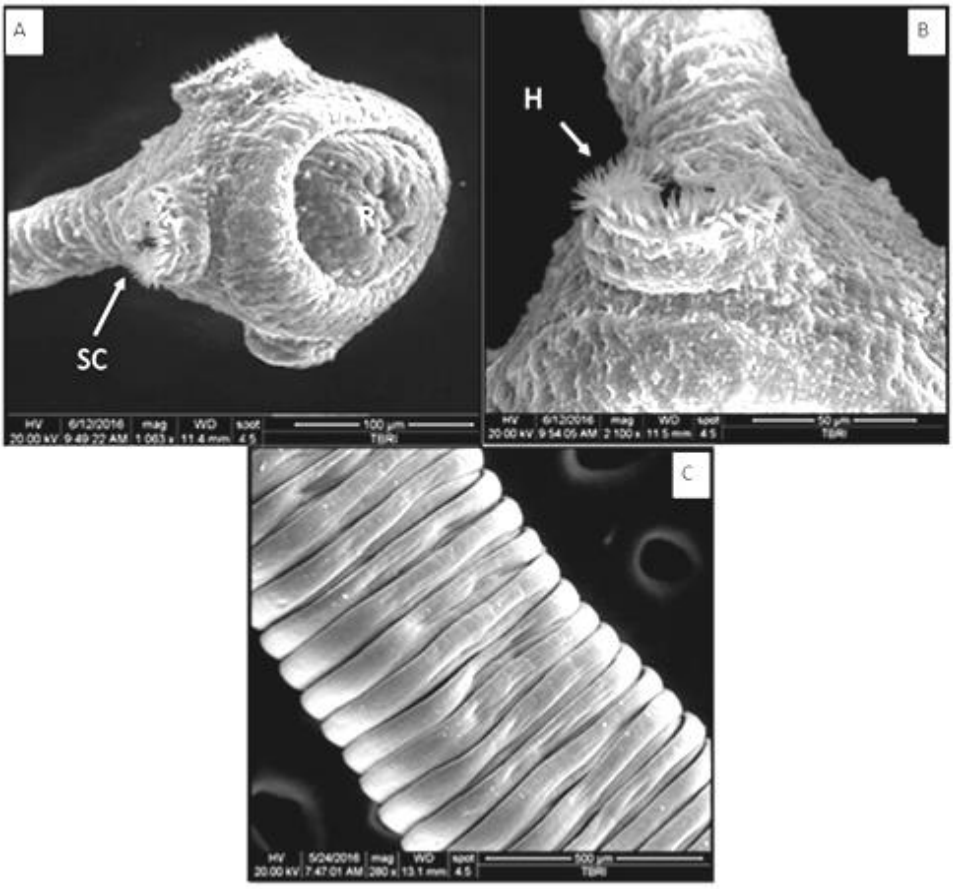

Figure (6): ESEM of *R. beveridgei* showing (A) Scolex with rostellum (R) and 3 of the 4 suckers (SC); (B) Scolex showing the hooks (H) of one sucker; (C) Body proglottids.

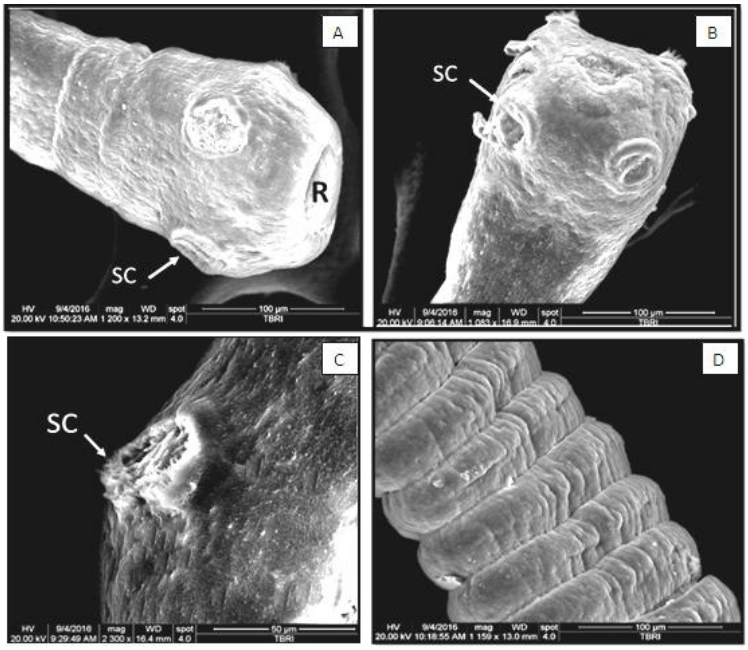

Figure (7): ESEM of *R. echinobothrida* showing (A and B) Scolex with rostellum (R) and 4 suckers (SC); (C) Scolex showing sucker (SC) with hooks; (D) Body proglottids.

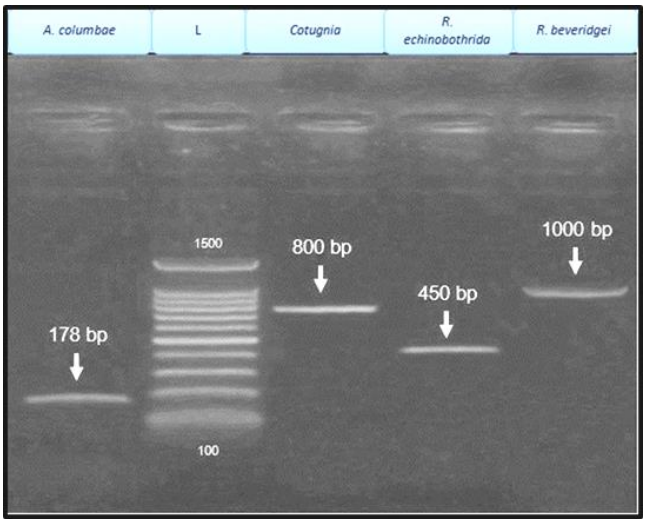

Figure (8): Electrophoretic mobility of PCR products of 18S rDNA gene for *Cotugnia polycantha*, ITS2 for the two *Raillietina* species and mt genome for *A. columbae* separated on 1.5 agarose gel. Molecular size of ladder (L) is 1500bps.

Table (2): Edited ITS2 and 18S rRNA genes of the three recovered species (*R. beveridgei, R. echinobothrida and C. Polyaca-ntha*).

Species	Gene sequence	Accession number
R. echinobothrida ITS2 gene	AGCGTGTGTGTGGCCTGGGGGTGCGCGTGTTGTGTGCAGGCCTCTCGGTGCTCTGGCTTCTTCCTAAGGT GTGGTCGCCTCAGGTGGCGTAGAGCTAGTGGTTGTGCCATGGCTGCAGTGTATACATGTGCACGTGTGAT ATATGTGCGCGTATATGTGTTGCGCTTGTGCGTGTGTGTGTGTACGTGTGTACGTGTGTGGGTAGCTG CATTTCACAAACCGTGCCCTAGTGTGCTGTGTGTGTTGATCTAACACTATGCGTGAAGTGTGCTACTGCT ACTGCTACCGCTGCTACGGGCGCCCCCATGAATCTGCTTGTGGTTGGTTAGAGTCATATCTGGCTCGTGG TGCGTGAGAGAGAAGAGAGAGAGAGTGGCCGCTATTTACATGTGA	**MF426021**
R. beveridgei ITS2 gene	CGACATCTTGAACGCATATTGCGGCCATAGGCTTGCCTGTGGCCACGTCTGTCCGAGCGTCGGCTTGTAA ACTATCACTGCGCGTTATAAGCAGTGGCTTGGGAGATTGCCGTGCAGTATGGCGGCCTGGTTGTCTCCGA TTATCACAGGCGTCGTCGCTTTAGACCATGTCCTCTCCACGCAATCTTTAAAGGTGTACGTGTGGGTGTG TGTATATTGGCTTTGTTAGCCTCTTATCCGTATCCATGCGTGCGTGCGTGCGTGCATGCGTGTGTGTGCG TGCGTGCGTGCGTGCGTGCCCGCCTCCCTACCGATGTGTGCATATTGCTTTGTGTGCGATTTGTGGGATT TGCGTGTACACATAGCGAGTAGGATGAGATGGGGCGCATTGCAGTGCGGCGCAACAGGATAGGATATTTG TTTATGGCTGAGCAAGTCGGTGCCTGCGTGGATGATGGGCGGTGGATGGGTGACGGATGAATGTGTGACT GAGACGTATTGGTGAGGCGTTTACTGCTGCGGCTTCTTCCTAATGTGTGGTCGCCTCTGGTGACGTCGAG CTGGTGGTTGTGCCATGACTACAGTATGTACATGTATATATTTGTGCATATGTATATGCGTGTGTGTGTG GGTGCGAATATATATGCATATGCACGTATACGTGTGCGCGTAGTTGCAGTTCACAAACCGTGGCTTAATG TGTATTGAGGGGTGCGCGAGTGGGTGTTATCTTGTGTGTGTGGGTGCATCCCTTGATGTATTATTATGTA GTACACGTAGAGTGAACTGTGGCTGCTGTGGTCAGTGTGTGCCGTGGTACATGCCGTGTCAACCGCCAGT CGTGAGGTAGGGCCAAATGCGCGTGTGTGGCATTTGTACTTGCTTCTTCTAATCTAATGGGGTCGAGCAA AATCTGGTTCATGTTNGTGTATTGATGTGCTGCTTTGTGGGTATTTATTGAATTCAAGAGCTAAGAGCCA AGAGAGTAGCTACCCTGACCT	**MF426022**
C. polyacantha 18S rRNA gene	CACTGTTCAAGCTCTACGGCCTGCCTTGAGCGCTCCNATTTTCTCAAAGTAAACGTTCAAGTCGCCAAGG ACACTCACCGAAGAGCATCCAGGACGATCTTGATCAAACAGGTGACGAGCGACGGAACGACTCCTGCTAG GACGACCGTCGTCGTCGACCGCAATCCAACTACGAGCGGTTTAACCGCAGCAACTTTAATATACGCTATT GGAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTGCCCTCCAATAGATTTAGCCGAGCTTATGTACGG CTCGGTCGTTCCGGTAACGCGTCTCATGGAGATACGTTCCGTTATTTTTCGTCACTACCTCCCCGATCCG GGAGTGGGTAATTTGCGTGCCTGCTGCCTTCCTTGGATGTGGTAGCCGTTTCTCAGGCTCCCTCTCCGGA GTCGAACCCTGATTCTCCGTTAACCGTTGTCACCATGGTAAGCACGTAGCGTACCATCGAAAGTTGATGA GCAAGTCATTTCAAAGATTCGTCGCCGGTGCTGGACCGTGCGATCAGCATAGTTATTCCGATTCACCAAC CTTTGGCGGACGAAGGCTAAGAGCCAACGCCCGATTGGTTTTGTACTAATAAATGCGCTCCGACGCGATT AGCGCCGAAGCTTCGAGGCATGTATTAGCTCTAGAATTTCCACAGTTATCCAAGTGGACATCAGTTCTTG CGAACAATGGCTGTTATAATGAGCCGTTCG	**MF426023**

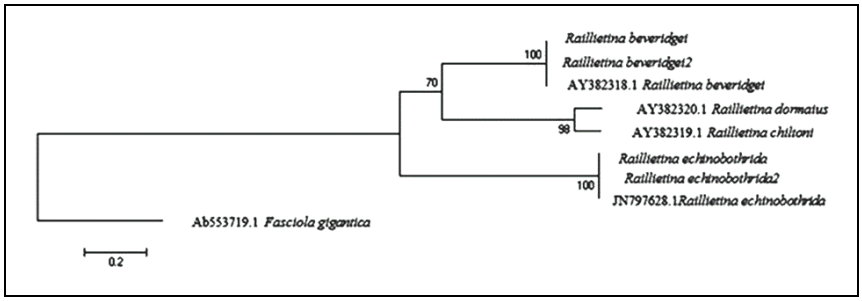

Figure (9): Maximum likelihood tree for the two samples of *R. beveridgei and R. echinobothrida* and other *related species of genus Raillietina* downloaded from GenBank. *Fasciola gigantica* (AB553719.1) was used as out group.

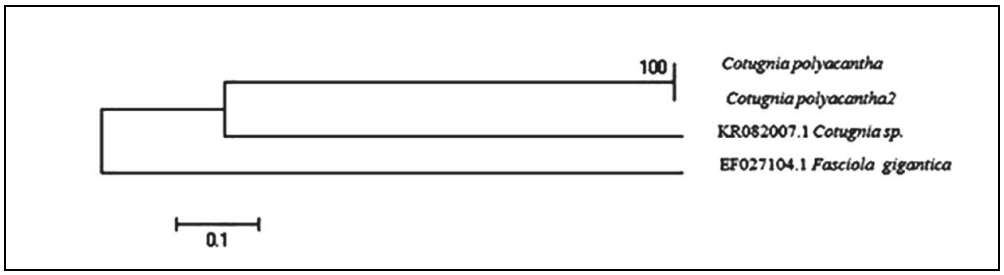

Figure (10): Maximum likelihood tree for the two samples of *C. polyacantha* and other *Cotugnia* downloaded from GenBank. *Fasciola gigantica* (EF027104) was used as outgroup.

DISCUSSION

In the present study light and environmental scanning electron microscope are used to determine the parasites surface structures since they are of great importance in the identification of the parasites on hand as mentioned by Ilie *et al.* (2008). *A. columbae* is characterized by having precloacal sucker provided with chitinized wall, spicules which are strong and equal or slightly subequal and 13 pairs of caudal papillae for male *Ascaris.* Tail is long, narrow and pointed, and the vulva aperture is nearly posterior to the middle of the body for females. These results are in accordance with those described by Banaja *et al.* (2013).

The present work showed that *C. polyacantha* are characterized by square-shaped scolex with four unarmed circular suckers and large oval retractile rostellum armed with hooks arranged in 2 rows, behind these hooks, numerous very flat spines take scale-like shape forming indentation at the base of rostellum which in an agreement with the descriptions mentioned by Bâ *et al.* (1995).

R. echinobothrida characterized by scolex armed with four circular suckers and oval large retractile rostellum armed with hooks arranged in 2 rows, mature segment with unilateral, single genital pores, these findings are in accordance with Ilie *et al.* (2008); Lalchhandama (2009); Shahin *et al.* (2011) and Waghmare *et al.* (2014). Also, *R. beveridgei* was characterized by scolex with four armed circular suckers, oval large retractile rostellum armed with hooks that arranged in 2 rows which is confirmed by the results found by O'Callaghan and Michael, (2004).

Molecular diagnosis as identification tools, among species, has been considered the most accurate and sensitive tools in recent years. Molecular markers based on DNA analysis which have been used for genetic characterization and identification of parasites especially helminths (Vilas *et al.*, 2005). Li *et al.* (2011) documented that DNA sequence provided an efficient method for species level identifications under the terms DNA barcoding. Also, DNA barcoding has become one of the most important scientific trends in the last decade according to Trivedi *et al.* (2015).

In the present study, the identification of *A. columbae* is confirmed by PCR amplification and sequencing of the mt genome, the same approach was adopted by liu *et al.* (2013) who used the mitochondrial genes to identify the 3 different species of *Ascaris*: *A. galli*, *A. columbae* and *Ascaridia sp.* The rDNA internal transcribed spacer (ITS) sequence data have revolutionized the phylogenetic analysis as a powerful tool in resolving remarkable taxonomic issues and discriminating genera and species across a large variety of organisms, because these regions ITS spacers are more variable and more informative than other parts of the rDNA locus (Coleman, 2003). Furthermore, the ITS2 markers have also been proposed for use in species barcoding and DNA microarrays, thus increasing the detection power of closely related species (Park *et al.*, 2007 and Engelmann *et al.*, 2009).

Molecular analysis of gene (ITS2) is capable to differentiate between the two *Raillietina* species *(R. beveridgei and R. echinobothridia*) that was collected from the intestine of domestic pigeon. Samples of *R. beveridgei* and *R. echinobothridia* of the present study was clustered only with their closely resembling sister species in GenBank with high bootstrap 100% which supported by Berry and Gascuel (1996) who stated that high bootstrap values close to 100% mean uniform support, if the bootstrap values for a certain clade is close to 100%, it means that nearly all the species of this clade have uniform characters and considered as a group.

The sequence of the data obtained in this study for *R. beveridgei* and *R. echinobothridia* ITS2 gene, was used successfully to construct a phylogenetic tree utilizing the related sequences available on the GenBank. The tree showed that our sequences were almost identical with the previous submitted sequences for the same species and highly related to *R. dormaious* and *R. chiltoni.* In turn the molecular analysis confirms the morphological identification of the samples of this study.

In the present study 18S rRNA was used for the identification of *C. polyacantha*; partial sequences of the same gene were subsequently used for the evaluation of phylogenetic relationships within several orders of the Eucestoda (Mariaux, 1998). The sequence of *C. polyacantha* 18S rRNA gene was used to construct a phylo-genetic tree using the related available sequences on GenBank it came out with one related sequence belong to *Cotugnia* sp. from India. From these results, *C. polyacantha*, collected from Egypt, is supposed to be the first to submit to GenBank. Molecular identification of species proved to be a very successful tool in separating morphologically similar species. Therefore, it is recommended to use molecular technique in helminths identification as the main methodological tool for accurate identification. Molecular technique was

capable to recognize hybrid and cryptic species, the technique is more accurate with the PCR primer being very specific to the species under investigation.

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التعريف الظاهرى و الجزيئى لبعض الديدان المعوية التى تصيب الحمامة المنزلية "كولومبا ليفيا دوميستيكا" من الإسماعيلية، مصر

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الملخص العربي

قد حددت الدراسة الديدان الطفيلية التي تصيب الحمامة المنزلية، كولومبا ليفيا دوميستيكا(*Columba livia domestica*) باستخدام الأساليب التقليدية (الميكروسكوب الضوئى والمجهرالبيئى الإلكتروني) وكذلك طرق البيولوجيا الجزيئية. تم شراء ١٢٠ حمام من الإسماعيلية بمصر خلال الفترة من ديسمبر ٢٠١٥ إلى نوفمبر ٢٠١٦. تم استخدام المجهر الضوئي و البيئي الإلكتروني لدراسة الخصائص الظاهرية العامة والسمات السطحية للطفيليات، وكذلك تم تأكيد هوية الديدان الطفيلية باستخدام تقنيات البيولوجيا الجزيئية من خلال الدراسة الحالية، تم تسجيل أربعة أنواع من الديدان الطفيلية؛ دودة خيطية (*Ascaridia columbae)* وثلاثة ديدان شريطية (*Cotugnia polyacantha*, and *Raillietina beveridgei, Raillietina echinobothrida*) وبلغت نسبة الاصابة بالديدان الطفيلية ٥٨.٣ %. هذا وقد تم إستخدام نتائج تتابع الحمض النووي (DNA sequencing) في تعريف وتقسيم الديدان الشريطية. وجديراً بالذكر أن هذه هي المرة الأولى التى يستخدم فيها *C. polyacantha* 18SrRNA فى تعريف هذا النوع من الديدان الطفيلية فى مصر وسوف يتم تسجيلها فى بنك الجينات. أيضا أظهر تفاعل ال PCR نتائج إيجابية فى تعريف الدودة الخيطية *A. columba*، ولهذا فمن الافضل استخدام التقنية الجزيئية في تحديد الديدان الطفيلية وخاصة في الأنواع ذات الصلة الوثيقة.