Optical, scanning electron microscope and Genetic Identification description of the tapeworm Ophryocotyle proteu (Fam: Davaineidae) in the pigeon Columbae livia

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Abstract
Davaineidae, is is a very large family and contains the most genus of Cestodes. Parasitize on vertebrates and among the fourteen genera registered under this family is the genus *Ophryocotyloides proteus*. The research materials consisted of cestoda isolated from small intestinal of *Columbae livia* from Najaf city in Iraq. The optical microscope and the scanning electron microscope revealed has large rostellum and the suckers are armed with small hooks. The genital openings are on one side. The vagina is located behind the lupus sac .To confirm the diagnosis cestoda, DNA was isolated and approximately 450 bp of the 18SrRNA gene was sequenced. The obtained DNA sequences were tabulated and then phylogenetic analysis was performed using the (MEGA X version) method.
Keywords: DNA identification, Ophryocotyle proteus , Columbae livia , 18S rRNA.

1. Introduction

Ophryocotyloides proteus is a parasite of birds. The rostellum contains hooks that are arranged in two circles, and the suckers are armed with small hooks arranged in concentric rows. The testicles are divided by the female glands into two groups on each side. The lupus cyst bypasses the excretory system and is not connected to it. The genital openings are on one side. The ovary is polar, non-polar, or bifurcated. Located behind the ovaries are the vitelline glands. The pregnant uterus is sac-like and extends behind the excretory system. The vagina is located behind the lupus sac, and it may be very wide. The seminal receptors are very small. These parasitic worms infect many birds [1].

life cycle: This worm has an indirect life cycle with many species of molluscs, genus Cepoea sp. Limax sp. , Agrilimax sp. , and Arion sp. They are intermediate hosts [2]. After the feces of birds containing eggs and pregnant segments come out, the embryos (oncospheres) are taken by intermediate hosts. Oncospheres develop in the intermediate host within 13 days at 26°C, or within 26-28 days at 15°C. Birds become infected by ingesting infected slugs, and within 10-13 days the worms reach adulthood [3]. Another cause of infection of birds with D. proglottina in New Zealand is molluscs of the family Limacidae and of an unspecified genus Agriolimacida.

2. Material and Methods

Sample collection: This study was conducted During February to June 2022, a total of 70 specimens of pigeon were *Columbae livia* captured and purchased from the store designated for the sale of birds. from Al-Najaf city in Iraq. Birds were examined for the cestode immediately or within a few hours of slathering.

Bird examination: After slaughtering the birds, they were brought to the laboratory of the Department of Life Sciences / College of Education for Girls. The examination process was carried out by dissecting the birds according to the method of [4] by opening the bird's body longitudinally using a very sharp scalpel from the compound passing through the abdomen and chest after removing its feathers. Conducting a macroscopic examination of the alimentary canal to note any damages that may be present on the outer surface of the alimentary canal of the bird, then the alimentary canal was separated from the body and placed in a petri dish containing saline solution (9%) at 37 °C to preserve it in its natural state, and examining the body cavity with a manual magnifying lens in search of parasites Or its larval stages, the alimentary canal was divided into four parts, which are the trachea, liver, esophageal bile sac, crop, gizzard, small intestine, large intestine, and the complex area, so that each part is separate, then each part was opened longitudinally by means of sharp scissors in a petri dish placed on a black background. After completing the process of opening these four parts, the process of examination and search for intestinal worms begins using a magnifying glass and a dissecting microscope to isolate large and medium-sized worms. As for small-sized worms, they are isolated using a fine needle.

Visual examination: After isolating the different worms, they were classified according to shape and with the help of a dissecting microscope, where their numbers were counted and kept in small plastic bottles containing 70% ethyl alcohol and glycerin after washing them with tap water to remove

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impurities and mucous materials attached to them.

Tapeworm isolation: The tapeworms extracted from the intestine of the palm tertulia seengalensis were removed and the tapeworms were washed with tap water, then placed in a solution of Vezlji, then I kept a section of worms in a bottle containing an ethical alcohol 70%, in addition to a few drops of kliserin for the purpose of fixing and later dyeing them for the purpose of diagnosing optical microscope. I preserved a section of worms in a bottle containing 10% until the electronic microscope was diagnosed. I preserved a section of worms in bottles containing absolute ethnic alcohol and placed in the strawberries until the molecular diagnosis [5].

Microscopic examination of tapeworms: After measuring the lengths of the tapeworms by means of a ruler, they were cut into suitable pieces and then dyed using the ready-made acetocarmine, where several drops of this dye were placed on the samples by means of a dropper in a watch bottle and with continuous examination of the dyed samples until they acquired the appropriate redness, and in the event that the sample acquired a dye It is shortened by adding several drops of hydrochloric acid (HCL) 10%. It is placed on the dye away from the model, after which the head is isolated from it. As for the rest of the dyed pieces, they were placed between two strips and tied using rubber bands and placed in 70% ethyl alcohol for 24 hours at room temperature.

After that, the compressed forms were opened and placed in ascending concentrations of alcohol: 70% (10-15 minutes), 80% (5-10 minutes), 90% (5 minutes), and then absolute alcohol (100%) for one minute, then into absolute alcohol and xylol at a ratio of (1:1) for one minute, then transferred to xylol for one minute to clarify the internal structures of the parasite. Finally, permanent segments of the parasite were prepared by loading it onto a slide using Canada balsam [6]. Pictures were taken with a digital camera that diagnosed the worms based on the diagnosis of the Natural History Museum and Research Center / University of Baghdad.

Scanning electron microscope examination of worms: Samples were examined at the University of Kufa / College of Science, supervised by Dr. Ahmed Hussein Muhammad Al-antaki. Whereas, after the drying process, the sample is loaded on the sample holders through a double - faced adhesive tape and then placed in the gold cover room that works to cover it by measuring the nanometer to keep the sample from damage due to the enormous energy from the electrons involved from the device. The samples are placed in their places to examine the use of Scanning electron microscope (SEM)/Inspect S50/FEI company. made in Netherland [7].

Molecular examinations

DNA extraction: DNA extraction was performed from worm samples (100 tapeworms) using a tissue DNA extraction kit (Tissue DNA extraction kit) prepared by Promega USA, and the extraction was carried out according to the company's instructions. The DNA was kept in the refrigerator until the PCR examination was performed.

Measure the concentration of the extracted DNA: The DNA extracted from worm samples was detected by using a Nanodrop spectrophotometer for detection and measuring the concentration of nucleic acids (DNA and RNA). By reading the absorbance at a wavelength ranging between 260-280 nm, the device was used as follows:

The purity of the extracted DNA samples was also determined by reading the absorbance using a Nanodrop Spectrophotometer at two wavelengths 260/280 nm, as the extracted DNA is considered pure when the absorbance ratio is (1.8).

PCR test method: A PCR assay was performed using primers for SrDNA genes (18) responsible for diagnosing tapeworms, Ophryocotyloides proteus [8], with the following steps:

1- Preparation of a PCR master mix primary mix: The polymerase chain reaction mixture was prepared using the 2 PCR Mix kit provided by Promega USA and according to the company's instructions, which is to prepare a multiplex polymerase chain reaction mixture in the PCR tubes equipped with the kit and containing the PCR components. Other components were added to the reaction mixture according to the company's instructions as in Table (1):

Table (1) Components of the PCR polymerase chain reaction mix

Volume	PCR master mix
5µL	DNA template
2µL	Forward primer (10pmol)
2µL	Reverse primer (10pmol)
12.5 µL	Micro litre master mix
3.5 µL	Nuclease free water
25 µL	Total

After completing the preparation of the PCR mixture, the tubes were closed and mixed carefully with a vortex homogenizer for 5 seconds. The tubes were transferred to a PCR Thermocycler to perform PCR Thermocycler conditions.

2- PCR Thermocycler conditions: The PCR test was carried out using a PCR Thermocycler, and the device was programmed as shown in Table (2):

Table (2) Thermal cycles of the PCR polymerase chain reaction

PCR Step	Repeat cycle	Temperature(C)	Time
Initial denaturation	1	95°	3min
Denaturation	35	95 °	30 sec.
Annealing		54 °	30 sec
Extension		72 °	1 min
Final extension	1	72 °	5 min
Hold	-	4	Forever

3- Gel electrophoresis: Electrophoresis was carried out using a 1.5% agarose gel to read the result of the

polymerase chain reaction (PCR) product analysis. After the completion of the migration process, the plate was quietly lifted from the basin and placed under a UV-transilluminator by exposing it to a wavelength of 260 nanometers to observe the bands of amplification products, then photographed.

3. Results and Discussion

Optical microscope description of the tapeworm Ophryocotyloides proteus isolated during the current study:

This type of tapeworm was isolated from the small intestine of Columbae livia. The worm body was 35 cm long and 5 mm wide. The head are 94 µm in diameter and contain four capsules with a diameter of 21 µm under 400x power and are equipped with one row of hooks 7 µm in length under 400x power. The head contain a rostellum with a diameter of 47 µm under 400x power, rostellum supplied with two rows of 9 µm long hooks under 400x power Figure (1), the mature segment is 14 µm long and 41 µm wide under a force of 400x Figure(2). The gravid segments contain eggs under a force of 400x , Figure(3) .

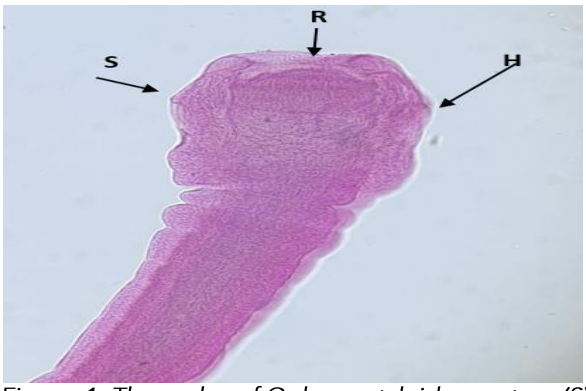

Figure. 1: The scolex of Ophryocotyloides proteus (S) suckers (R) Rostellum (H) hooks, ***Columbae livia host****, magnification 400x*

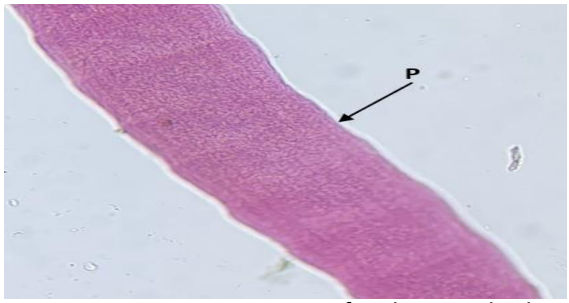

Figure 2: Mature segments of Ophryocotyloides proteus (P) genital pore , ***Columbae livia*** *host. 400x magnification power*

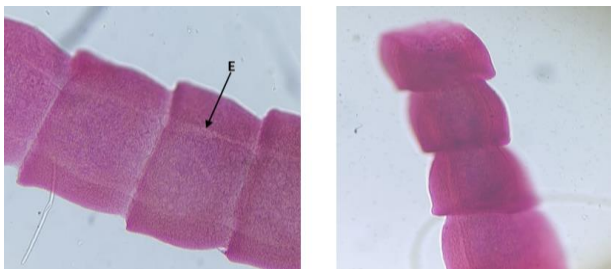

Figure 3: The gravid segments of Ophryocotyloides proteus (E) eggs. ***Columbae livia*** *host. magnification 400x.*

A scanning electron microscope described the isolated Ophryocotyloides proteus.

During the current study: The scanning electron microscope revealed that the head of Ophryocotyloides proteus are almost oval in shape and contain four round-shaped receptacles with a diameter of 27 micrometers and are armed with a row of hooks with a length of 8 micrometers. The hooks are surrounded by a circular band with a width of 4 micrometers that contains many flat spines that resemble scales and show bilateral symmetry and have a slightly convex appearance (1) Bar=100µm, (2) Bar=400µm Figure (4), Bar=100µm Figure (5) The Mature segments is rectangular in shape and superimposed one on top of the other.The length of the mature segments is 0.12 mm and its width is 0.40 mm.(1) Bar=300µm ,(2) Bar=200µm Figure (6). The gravid segments are longer than their width, and the end of each piece is separated by the waist from the piece that comes after it. The length of the piece is 0.66 mm and its width is 0.36 mm,and contain eggs ,(1)) Bar=400µm, (2) Bar=100µm Figure (7).

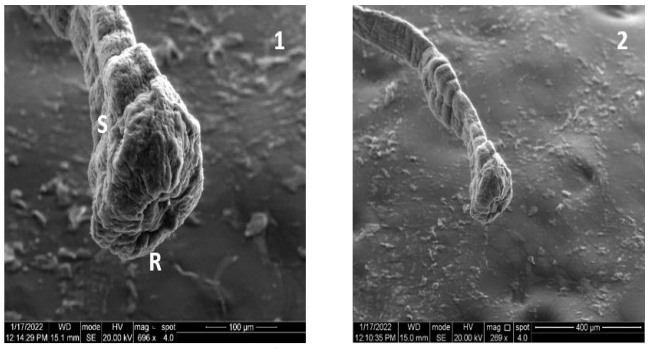

Figure 4: The scolex of Ophryocotyloides proteus (S) suckers (R) Rostellum, ***Columbae livia host*** *(1) Bar=100µm, (2) Bar=400µm.*

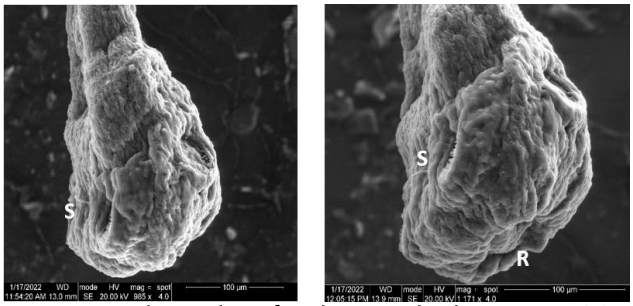

Figure 5: The scolex of Ophryocotyloides proteus (S) suckers (R) Rostellum, Columbae livia host, Bar=100µm,

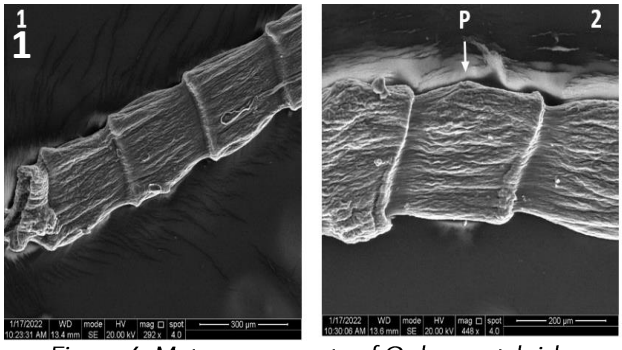

Figure 6: Mature segments of Ophryocotyloides proteus, (P) genital pore, Columbae livia host, ,(1) Bar=300µm , (2) Bar=200µm

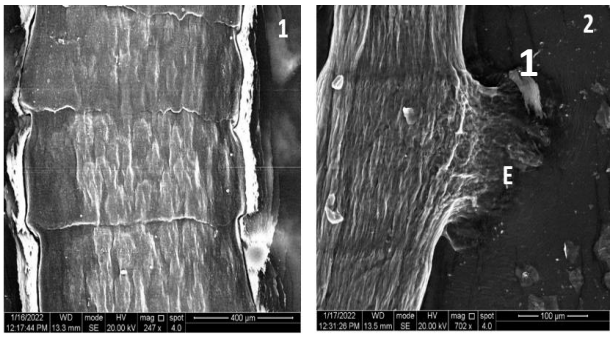

*Figure 7: : The gravid segments of Ophryocotyloides proteus. (E) eggs. **Columbae livia** host,(1)) Bar=400µm, (2) Bar=100µm*

Genetic diagnosis of the tapeworm, Ophryocotyloides proteus isolated during the current study

Identification of Ophryocotyloides proteus by PCR technique, and electrophoresis of the PCR product of the 18SrRNA gene in the intestine of mutating pigeons. In Figure (8). In row (1-7) are positive samples of seven 450 bp isolates.

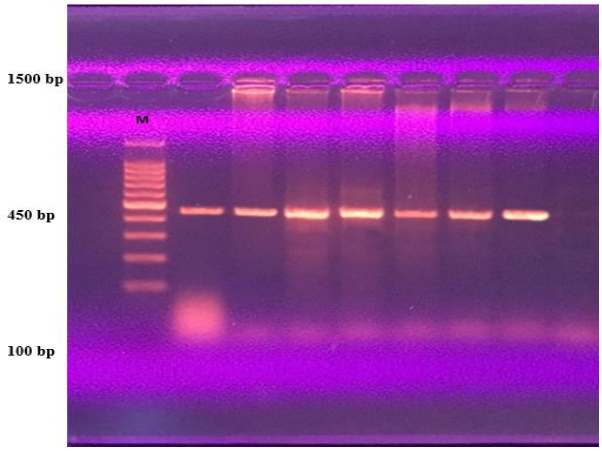

Figure 8: Agarose gel electrophoresis image showing the PCR product of 18SrRNA analysis of Ophryocotyloides proteus isolated from columbia livia bowel samples. M (Marker ladder 1500-100 bp). In rows (1-7) is a positive Ophryocotyloides proteus with a size of 450 bp.

Light and electron microscope examination of the tapeworm, Ophryocotyloides proteus in this current study showed that Ophryocotyloides proteus differs from Ophryocotyloides dasi [9], where the head was found to be 378 mm in diameter and separated by a constriction from the rostellum. The eye suckers are oval or circular in shape, with a diameter ranging between 185-195 mm, and are muscular and armed with approximately 10 rows of dense hooks, 12-14 mm long. The rostellum disc has a diameter of 185 mm and is armed with two rows of hooks, 16.5-17.5 mm long in both rows. The genital openings They are unilateral, located 26-27% from the edge of the lateral body segment opposite the caecum, pear-shaped, thick-walled, the uterus is a barely visible sac, no egg capsules and calcareous particles are present in the last chordal segment [10].

Observations showed that the worm Ophryocotyloides proteus is similar to that found by Ophryocotyloides [11] which parasitizes the order Piciformes as the genus Psilopogon and differs from it in the absence of egg capsules only [12].

The present study showed that Ophryocotyloides proteus differs from Ophryocotyloides barbeti [13] of the brown-headed bird host Psilopogon zeylanicus and O. haemacephala [13] in the host of the scarlet-breasted bird Psilopogon haemacephalus in that it The head are larger in diameter, and their eyelids are also larger in diameter, and the hooks are (23-29) long, and the muzzle is larger in diameter (112-170) [13]. The current study is the first study of the tapeworm, Ophryocotyloides proteus, in Iraq.

4. Conclussions

Tapeworms Ophryocotyloides proteus, It infects the small intestine of Columbae livia , and by visual examination, we find that this infection led to severe inflammation and bleeding. It was detected by light microscopy and scanning electron microscopy.The morphological characteristics of this worm showed that it has a large rostellum and the suckers are armed with small hooks in head, The Mature segments has genital openings are on one side. The vagina is located behind the lupus sac. The gravid segments are longer than their width and contains eggs. DNA was isolated and approximately 450 bp of the 18SrRNA gene was sequenced.

References

Yamaguti, S. (1961). Systema helminthum. Volume III. The nematodes of vertebrates.

Systema Helminthum. Volume III. The Nematodes of Vertebrates.

Abdou, A. H. (1958a). Studies on the development of *Davainea* proglottina in the

intermediate host. The Journal of Parasitology, *44*(5), 484–488.

Abdou, A. H. (1958b). The Life-Cycle Of *Davainea* Progottina Davaine And Relations

Between The Proglottids Discharged Daily And The Number of Tapeworms In The

Domestic Fowl. Canadian Journal of Comparative Medicine and Veterinary Science,

22(10), 338.

Al-Hussaini, A. H. and Demian, E. S. (1982). Practical Animal Biology. Vol. II, 11TH . ED . DAR AL-MAAREF, 283–299.

Al-Shaibany, K. T. (2008). Isolation and identification of Ectoparasite and helminthes

parasitic in digestive system of rock pigeon *Columba livia* (Gmelin 1789) in AL-Diwaniya

city. Iraq. M. Sc.Thesis, Coll. Edu. Univ. AL-Qadisiya.

Al-saadi, A. A. J.; Abdul-Hadi, W. H. and Abdullah, A. H. (2017). Histopathological Study

of Infection with Parasitic Intestinal Helminthes on Passer domesticus in Tikrit City, Iraq.

Ibn AL-Haitham Journal For Pure and Applied Science, 29(1).

Morris, J. K. (1965). A formaldehyde glutaraldehyde fixative of high osmolality for use in

electron microscopy. J. Cell Biol, 27, 1A-149A.

Sambrook, J. and Russell, D. W. (2006). The condensed protocols from molecular cloning: a laboratory manual (Issue Sirsi) (i9780879697723).

Tandan, B. K. and Singh, K. S. (1964). *Ophryocotyloides dasi* sp. n.(Cestoda: Davaineidae) from a Himalayan barbet. Zoologischer Anzeiger, 173(6), 441–443.

Mariaux, J. and Georgiev, B. B. (2020). Cestode parasites (Neodermata, Platyhelminthes) from Malaysian birds, with description of five new species. European Journal of Taxonomy, 616.

Fuhrmann, O. (1909). Neue Davaineiden.

Movsesyan, S. O. and Beer, S. A. (2003). Davaineata—Tapeworms of Animals and Man. Osnovy Tsestodologii, 13(Part 1), 1–395.

Singh, K. P. (1960). Some avian cestodes from India. I. Species belonging to families Davaineidae and Biuterinidae. Indian Journal of Helminthology, 11(1/2), 1–24.