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Morphological and histopathological studies of *Thelandros chalcidae* (Oxyuroidea: Pharyngodonidae) infecting *Chalcides ocellatus* from Egypt

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Abstract

Background: *Thelandros* (Pharyngodonidae) is a gastrointestinal nematode parasite with a life cycle including lizards as main hosts. *Thelandros chalcidae* collected from the large intestine of the Egyptian ocellated skink, *Chalcides ocellatus* were described and illustrated by light and scanning electron microscopes. Seven out of fifteen (46.66%) of the examined lizards were found to be naturally infected. Also, host intestinal tissues were evaluated from hematoxylin/eosin-stained sections to describe any histopathological changes.

Results: Microscopic examinations revealed that the recovered pharyngodonid species characterized by mouth with triangular opening and surrounded by six simple lips, the cuticle had regular transverse annulations extending from the posterior margin of the lips to the end of the body. Male was cylindrical with distinct truncated posterior end and measured 1.59–1.86 (1.64 ± 0.10) long and 0.29–0.37 (0.32 ± 0.01) in maximum width at the level of mid-body. Female measured 1.72–2.43 (1.85 ± 0.2) long and 0.36–0.49 (0.42 ± 0.01) maximum width at the mid-body level, terminated posteriorly in a short, stout spike. Histological studies observed structural alterations represented by leukocytic infiltration, villi atrophy, and muscularis degeneration. These changes were indicative of inflammatory and degenerative reaction due to *Thelandros chalcidae* infection.

Conclusion: The present morphological study revealed that the recovered pharyngodonid species was *Thelandros chalcidae* causing pathological alterations in *Chalcides ocellatus* intestinal tissues.

Keywords: *Thelandros*, *Chalcides ocellatus*, Pharyngodonidae, Microscopic examination, Histopathology

Background

There are more than 400 species of reptiles occur in Egypt. One of the most abundant and widespread reptiles in Africa and Egypt is the lizard, *Chalcides ocellatus* (Al-Deen et al., 1995). Unfortunately, little consideration has been given to their helminths community and few records exist in the literature (Al-Deen & Al-Shareef, 1996; Ibrahim & Soliman, 2005). Genus *Thelandros* was established by Wedl (1861) for *T. alatus*, a nematode from

the intestine of an Egyptian mastigure, *Uromastix aegyptia*. The difference between *Parapharyngodon* (Chatterji, 1933) and *Thelandros* (Wedl, 1861) was questionable based on morphological similarities. Some authors considered them as synonym (Baylis, 1936; Karve, 1938; García-Calvente, 1948; Skryabin et al., 1951). However, Freitas (1957) considered them as different genera likewise (Adamson, 1981; Baker, 1987; Hering–Hagenbeck et al., 2002; Sharpilo, 1976). Although, previous studies recorded *Thelandros* sp. infection in wild animals from Egypt (Abdel-Ghaffar et al., 2018, 2020; Ibrahim et al., 2005; Rabie et al., 2012); the taxonomic status of some

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species is still under discussion and no pathological studies have been undertaken to assess the health impact on their hosts. Therefore, our aim was designed to study the morphology of *Thelandros chalcidae* and its impact on *Chalcides ocellatus*.

Materials and Methods

Worm collection

Fifteen Egyptian lizards, *Chalcides ocellatus* were collected from Abu Rawash region, Giza, Egypt from May to September 2020. Lizards were transported alive to the laboratory, they were euthanized with an overdose of sodium pentobarbital, subsequently necropsied and examined for helminths. The intestinal contents were examined for helminths under a stereomicroscope (OLYMPUS, SZ51). The collected worms were washed several times in saline solution then preserved in 70% ethanol.

Microscopic examination

The recovered nematodes were transferred to lactophenol for clearance, then examined and photographed using light microscope (LEICA DM 750) supplied with a LEICA ICC 50 HD Camera. For scanning electron microscopy, the worms were fixed in a solution of 3% glutaraldehyde, washed in 0.1 M sodium cacodylate buffer (pH 7.4), dehydrated through a graded ethanol series (50%, 60%, 70%, 80%, 90% and 100%), and then dried at 30°C for 30 min using critical point drier "LEICA, EM CPD300". After complete drying, nematodes were mounted on SEM stubs, coated with gold and examined with JEOL JSM-5200 SEM (Tokyo, Japan) at 25 kV as accelerating voltage. All measurements (mean \pm SD, followed by a range in parentheses) are in millimeters (mm).

Histopathological examination

For histological studies, target organ (large intestine) of the Egyptian lizards, *Chalcides ocellatus* were carefully isolated and washed out in saline water then fixed in 10% buffered formalin. Tissues were dehydrated and processed for paraffin wax embedding. Sections were cut by a rotary microtome and stained with hematoxylin and eosin, then examined for any histopathological lesions and photographed using LEICA DM 750 microscope supplied with a LEICA ICC 50 HD Camera.

Results

The helminthological examination of 15 ocellated skink *Chalcides ocellatus* revealed natural infection by *Thelandros chalcidae* with a prevalence of (7/15) 46.66%. The mean intensity of infection was 1–3 parasite per host.

Description (Fig. 1a–i)

Generally

The body was robust cylindrical, whitish in colour, tapering at both ends with prominent cuticle striations from cephalic extremity and continuing to posterior end. Oral opening surrounded by 6 small lips. The esophagus was muscular, cylindrical ending by a well-developed bulb which leads to the intestine. Small lateral alae present. Prominent sexual dimorphism.

Male

Small, cylindrical body, distinctly truncated posteriorly and measured 1.59–1.86 (1.64 ± 0.10) mm long and 0.29–0.37 (0.32 ± 0.01) mm in maximum width at the level of mid-body. Oral opening surrounded by 6 small lips. Length of esophagus (including bulb) was 0.52–0.61 (0.57 ± 0.02) mm long and 0.039–0.055 (0.043 ± 0.001) mm wide. Small lateral alae reaching the tail in an auricular form and being widest at the cloacal level. The testes at mid-body, genital cone simple with papillae disposed outside this cone with single spicule measured 0.032–0.037 (0.035 ± 0.001) mm long. Gubernaculum absent. The tail filament was narrow, sharply pointed, inserted dorsally, terminal in position and measured 0.14–0.22 (0.17 ± 0.01) mm in length and 0.028–0.034 (0.03 ± 0.001) mm in width.

Female

Cylindrical body measured 1.72–2.43 (1.85 ± 0.2) mm long and 0.36–0.49 (0.42 ± 0.01) mm maximum width at the mid-body level, tapering anteriorly to blunt point and posteriorly ending in short, stout spike. Oral opening surrounded by 6 small lips. Esophagus was 0.58–0.69 (0.65 ± 0.02) long and 0.041–0.065 (0.045 ± 0.001) mm wide. Nerve ring 0.120–0.143 (0.126 ± 0.01) mm from the anterior end. Vulva located at the mid-body. Ovary amphidelphic, the anterior ovary extended to excretory pore level and the posterior ovary extended posteriorly to anal opening. Uteri divergent. Anus slit like located at 0.27–0.34 (0.3 ± 0.01) mm from the posterior end. The posterior extremity of the body extends like a very short smooth tail measured 0.063–0.11 (0.08 ± 0.01) in length.

Taxonomic summary

Type species: *Thelandros chalcidae* (Abdel-Ghaffar et al., 2020) (Family: Pharyngodonidae).

Type-host: Ocellated skink *Chalcides ocellatus* (Family: Scincidae).

Site of infection: Large intestine.

Type-locality: Abu Rawash area, Giza Egypt.

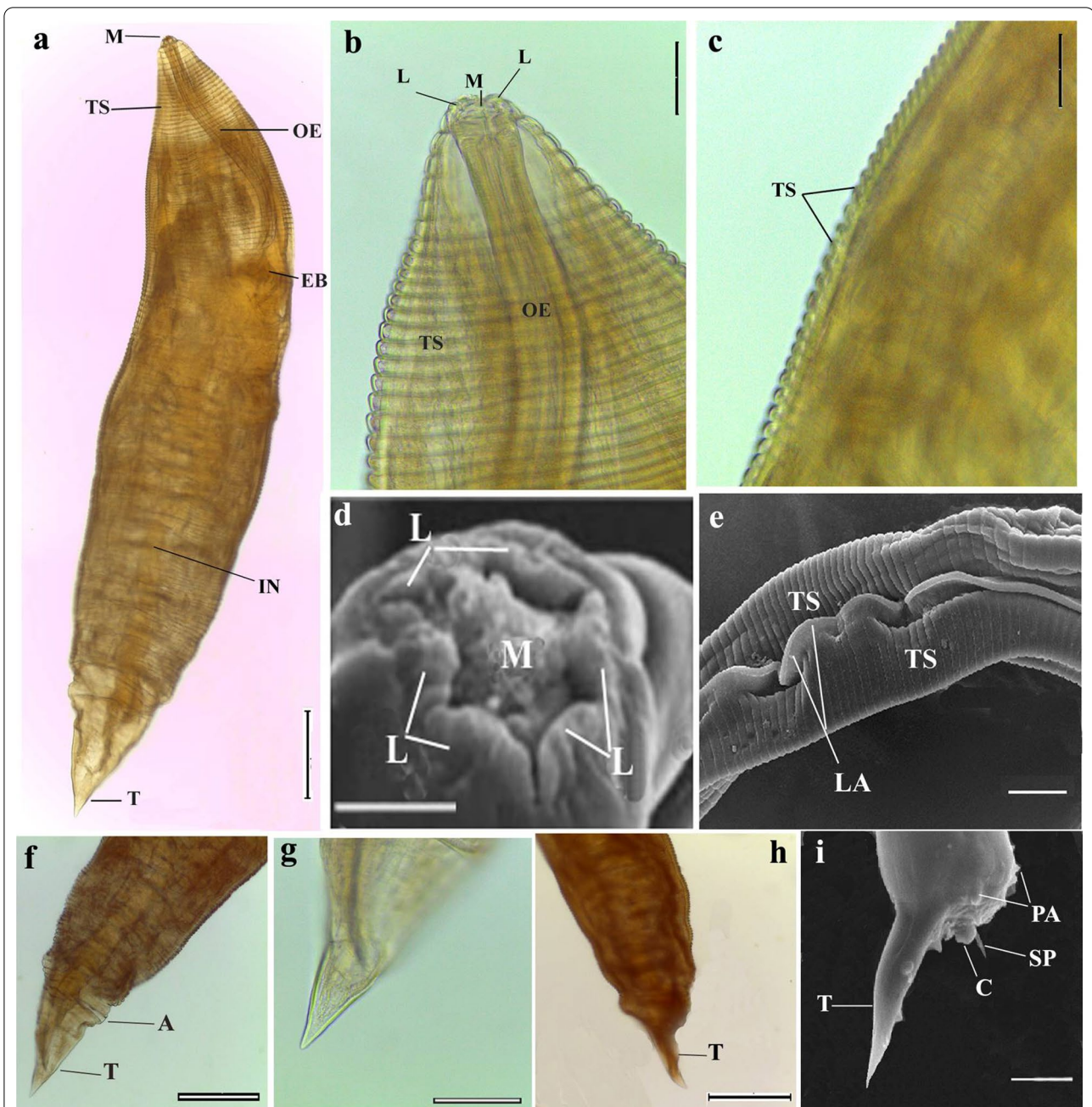


Fig. 1 a–c, f–h Photomicrograph of *T. chalcidiae* showing a Whole worm with mouth (M) followed by esophagus (OE) with esophageal bulb (EB) lead to long intestine (IN), cuticle with transverse striations (TS), scale bar = 200 µm. b Anterior extremity showing mouth (M), lips (L), esophagus (OE) and transverse striations (TS), scale bar = 50 µm. c High magnification showing cuticle with transverse striations (TS), scale bar = 5 µm. f Posterior extremity of female worm with tapered tail (T), anus (A), scale bars = 200 µm. g High magnifications of female tail region, scale bars = 50 µm h Posterior extremity of male worm with tail (T), scale bars = 200 µm. d, e, i Scanning electron micrographs of *T. chalcidiae* d Cephalic region showing mouth (M) with six lips (L), scale bar = 50 µm. e Midbody showing transverse striation (TS) and lateral alae (LA), scale bar = 50 µm. i Truncated posterior extremity of male worm with pointed tail (T), single spicule (SP), caudal papillae (PA) and cloaca (C) scale bar = 50 µm

Prevalence and Intensity of infection: (7/15) 46.66%, 1–3 respectively.

Histopathological findings

Sections of the intestinal tissue revealed cellular infiltration around the embedded worms which peripherally displaced the muscle fibers. Furthermore,

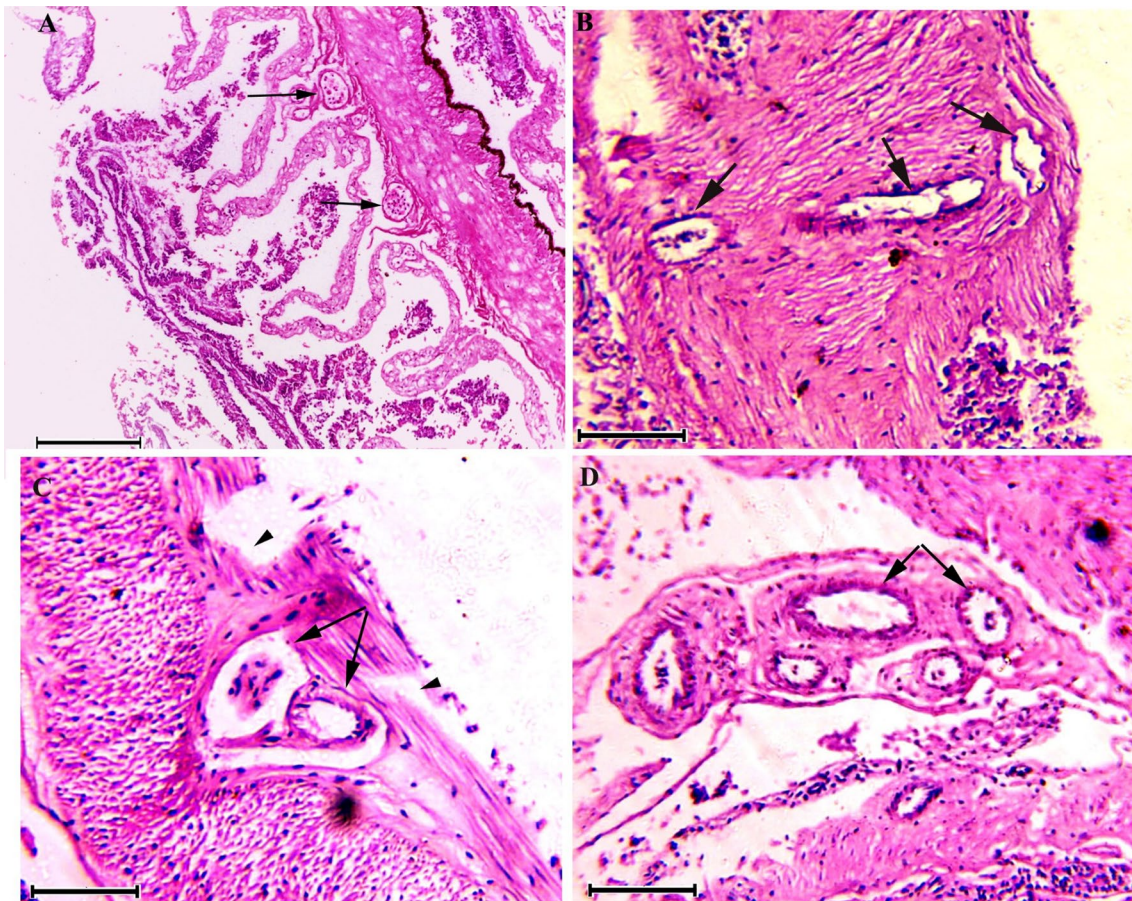


Fig. 2 Cross sections of intestine of *C. ocellatus* naturally infected with *T. chalcidiae* showing: **a** Parasites penetrate along different intestinal layers. **b** Embedded worms which displaced the muscle fibers **c** Disintegration and erosion of muscularis and serosa layers with severe lymphocyte infiltration, **d** Fibrosis in connective tissue, degeneration and atrophy of villi. Arrowhead: erosion and disintegration, Arrow: burrowing of the parasites, scale bars = 200 μ m

moderate fibrosis in connective tissue was evident around this area, and severe lymphocyte infiltration (Fig. 2A, B), erosion of the muscularis layer, lesions associated with severe fibrosis and an inflammatory response reached serosa layer. Finally, the effects were varied and distributed among the four layers of the intestine leading to disintegration of muscularis and serosa layers due to burrowing of the parasite (Fig. 2B, C) as well as degeneration and atrophy of villi, infiltrated inflammatory cells diffused in the lamina propria, hyperplasia of the mucosal glands were observed (Fig. 2D).

Discussion

Oxyurids are parasitic nematodes infecting reptiles that constitute a very interesting group although their knowledge remains scarce (Aho, 1990; Bush, 1990; Martin & Roca, 2004; Sharpilo et al., 2001). Species of genus *Thelandros* Wedl (1861) infect many reptilian hosts (Burse & Goldberg, 2005; Dung et al., 2009).

Walton (1941) reviewed the geographical and host distribution of the genus *Thelandros* and pointed out that many of the known species for this genus were recorded from North Africa. Petter and Quentin (1976) considered *Parapharyngodon* a synonym of *Thelandros*. However, *Thelandros* sp. differentiated from *Parapharyngodon* sp. based on the posterior end morphology in both sexes (Burse & Goldberg, 2005) *Parapharyngodon* sp. males do not have a conical-shaped genital area, or an accessory

piece. In contrast, *Thelandros* sp. males have a prominent genital cone with papillae disposed outside this cone and caudal appendage inserted sub terminally on the body (Bursey & Goldberg, 1999; Bursey et al., 2013). Females of *Parapharyngodon* sp. generally have a conical shaped tail with a thick pointed end, like a spike. However, *Thelandros* sp. females have varied tail morphology (Bursey et al., 2013; Ramallo et al., 2016; Velarde-Aguilar et al., 2015).

Bursey and Goldberg (2005) allocated 30 species to the genus *Thelandros* and stated the main generic features for *Thelandros* species based on the pattern of the caudal papillae, spicule length and morphology of the anterior cloacal lip in males, location of the vulva and morphology of the posterior end of the female. The current pharyngodonid species revealed main features of genus *Thelandros* represented by tapering at both ends with prominent cuticle striations from cephalic extremity and continuing to posterior end. Male has a conical-shaped genital area with papillae disposed outside this cone and distinct truncated posterior end with single spicule while female posterior extremity extending in short, stout spike.

Thelandros species herein, resembles *T. micipsae* Seurat (1917); *T. scleratus* Travassos (1923) and *T. luciysi* Hering-Hagenbeck (2001) and *T. chaicidae* Abdel-Ghaffar et al. (2020) by the absence of gubernaculum and caudal papillae surrounded the genital cone and similar to the latter one by presence of long lateral alae reach from the cephalic region to the posterior extremity and male spicule. Also, *T. awokoyai* Babero and Okpala (1962); *T. arequipensis* & *T. yurensis* Calisaya and Cordóva (1997); by the presence of short tail; *T. markovi*, *T. galloti*, *T. balanchardi* Caballero (1968); *T. ortleppi* Petter (1966); *T. samburuensis* Bursey and Goldberg (2005) by possessing smooth cloacal lip. However, differ from *T. kuntzi* Delle (1954); *T. luciysi* Hering-Hagenbeck (2001) by absence of lateral alae and *T. aegypti* Rabie et al. (2014) by possessing oral opening without lips and absence of male spicule. Additionally, the recovered parasite sharing *T. alatus* Ibrahim et al. (2005); *T. amburuensis* Bursey and Goldberg (2005); *Thelandros* sp. Hering-Hagenbeck et al. (2002); *T. vietnamensis* Dung et al. (2009); *Thelandros* sp. Rabie et al. (2012); *T. aegypti* Rabie et al. (2014) and *T. chaicidae* Abdel-Ghaffar et al. (2020) in host type as well as site of infection and the latter three by having the same geographical location.

Oxyurids live in the stomach or intestines of all classes of vertebrates; attach to the mucosa cause a wide range of damages including tissue degradation, hemorrhage, inflammation and mesenteric visceral adhesions (Botella & Esteban, 1995; Mohammed et al., 2017; Naem et al., 2006). The present histological studies revealed numerous pathological alterations in different layers of the large

intestine of *Chalcides ocellatus* infected with *T. chaicidae* in agreement with previous observations by Schaftenaar et al. (2000) who reported pathological changes in lizard intestines due to nematodes infection. Also, in the present study, complete destruction of intestinal layers represented by erosion of the surface epithelium reaches the muscularis and serosa layers with severe leucocytes infiltration and lesions due to burrowing of the parasite, in consistent with previous reports by Dunn et al. (1983); Botella and Esteban (1995) and Meguid and Eure (1996) who reported that the parasites may occasionally change their site of attachment causing erosion of surface epithelium reaches as deep as the muscularis mucosa results in total loss at the region of attachment. Additionally, histological examination revealed atrophy in intestinal villi, this state may be due to the feeding of parasites on the villi, leading to its disappearance and the lack of surface area of absorption and thus low efficiency of absorption of nutrients and mineral salts (Dunn et al., 1983; Molnar et al., 1994).

Conclusions

Based on morphological and morphometric evidence, the current parasite resembles *Thelandros chalcidiae*, as well as sharing the same host species, site of infection, and locality record. As a result, we can mark it as *Thelandros chalcidiae*. Furthermore, for the first time in Egypt, we focused on the pathological effects of *T. chalcidiae* on *Chalcides ocellatus*.

Abbreviations

A: Anus; C. *ocellatus*: *Chalcides ocellatus*; C: Cloaca; EB: Esophageal bulb; IN: Intestine; L: Lips; LA: Lateral alae; M: Mouth; OE: Esophagus; PA: Caudal papillae; SP: Spicule; T: Tail; *T. chalcidiae*: *Thelandros chalcidiae*; TS: Transverse striations.

Acknowledgements

This work is supported by Faculty of Science, Cairo University, Egypt. Authors extend their appreciations to members of Zoology Department in helping to complete this work.

Authors' contributions

MF and NM have provided guidance during development of idea and prepared different figures required, wrote and revised the manuscript. Both authors read and approved the final manuscript.

Funding

None.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

All animal procedures were carried out according to the regulatory laws regarding experimental Animal Ethics Committee, Faculty of Science, Cairo University, Egypt (CU-IACUC). The informed consent obtained from this study was written and received ethical approval number: (CU/1/S/54/17).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests related to this study.

Received: 7 July 2021 Accepted: 4 November 2021

Published online: 18 November 2021

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