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Redescription and Molecular Identification of *Proctotrema prominens (Monorchiidae: Odhner, 1911*) from *Parupeneus forsskali,* a Red Sea Goatfish in Egypt

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Abstract: *Proctotrema prominens*, a trematode parasite originally identified by Wee, Cribb, and Cutmore in 2022 and belonging to the Monorchiidae family described by Odhner in 1911, was recovered from the intestinal tract of the Red Sea goatfish, *Parupeneus forsskali*, in the Red Sea region of Egypt. The prevalence rate of the parasite was found to be 42.6%, with a distribution of 51.5% in males and 34% in females. Morphologically, the parasite displayed a slightly fusiform body with a funnel-shaped oral sucker larger than the ventral sucker, a single testis, a cylindrical cirrus sac, a smooth ovary, an unspined genital atrium, caeca extending posteriorly into the post-testicular zone, and terminating near the posterior extremity. Molecular techniques utilizing IST2 rDNA facilitated the accurate identification of the parasite. Comparative analysis of the IST2 sequence against available monorchiids on GenBank confirmed the parasite's classification within the *P. prominens* clade. This study represents the first redescribed instance of *P. prominens* from a novel fish host, *Pa. forsskali*, of the Mullidae family described by Rafinesque in 1815, discovered in the Red Sea near Safaga, Egypt, marking a new geographical locality for this species.
Keywords: Red Sea fish, *Proctotrema prominens*, *Parupeneus forsskali*, IST2 rDNA, Egypt.

1. Introduction

The Monorchiidae family, described by Odhner in 1911, comprises a diverse group of trematodes that parasitize the intestines of marine fishes. Molecular tools have been instrumental in classifying these parasites within the Plagiorchiida order [**1, 2**]. Members of the Monorchiidae family are distinguished by their complex terminal genitalia adorned with specific spines and a spiny tegument [**3**].

The genus *Proctotrema*, also described by Odhner in 1911, encompasses eight recognized species, with four known to parasitize fish from the haemulid family and the remaining four from the atherinopsid, eleginopid, mullid, and sparid families [**4**]. These trematodes are characterized by a single testis situated in the anterior half of the hind body and vitelline follicles located between the posterior edge of the ventral sucker and the anterior edge of the ovary [**5**].

Among the host fishes, goatfishes, particularly *Parupeneus forsskali* (Perciformes: Mullidae), hold significant economic and commercial importance in the northern Egyptian Red Sea region [**6**]. Previous research has documented the presence of *P. prominens*, identified by Wee, Cribb, and Cutmore in 2022, parasitizing *Pa. forsskali* in the Red Sea waters of Egypt, marking the first record of this species in the region.

2. Materials and methods

2.1. Morphological Data:

A total of 68 fish samples (33 male and 35 female) were collected from Safaga in the Red Sea of Egypt between April 2019 and May 2021. The fishes were captured and immediately transported to the Parasitology Laboratory, Zoology Department, Faculty of Science, Sohag University, Egypt. Fish identification was conducted based on established criteria [**7, 8, 9**], and further confirmation was obtained from information available on the FishBase website [**10**].

The gastrointestinal tract was carefully untangled, and the entire digestive system along with other viscera was longitudinally opened. Macroscopic and microscopic examinations of various organs were performed to detect any trematode parasites. The collected parasites were cleaned by washing them several times with an isotonic saline solution of 0.9% [**11**]. Encountered trematodes were flattened between two coverslips gently tied together with a thread, fixed in A-F-A solution (Alcohol-formal-acetic) [**12**], and then preserved in 70% Ethanol until staining with acetic acid alum carmine [**13**]. Subsequently, specimens were dehydrated in ascending concentrations of ethanol, cleared in clove oil, mounted in DPX, photographed, and drawn using a camera lucida. Measurements were expressed in millimeters (mm), and samples were identified using a key for vertebrate trematode parasites [**14**].

2.2. Molecular Data

Total genomic DNA was extracted using the QIAamp DNA Mini Kit (Catalogue No. 51304). A 470-base pair (bp) region of the ITS2 nuclear ribosomal DNA was targeted for amplification using the forward primer (5'-GGTAC CGGTGGATCACTCGGCTCGTG-3') and the reverse primer (5'-GGGATCCTGGTTAGTTTCTTTTCCT CCGC-3') [15]. PCR amplifications were performed using the Emerald Amp GT PCR Master Mix Kit (Takara) (Code No. RR310A) in a total volume of 25 µl, consisting of Emerald Amp GT PCR Master Mix, 1 µl of each primer, 5 µl of DNA template, and 5.5 µl PCR-grade water. The PCR amplification was carried out in a thermal cycler (Eppendorf) with the following program: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 40 sec, extension at 72°C for 45 sec, and final extension at 72°C for 10 min [16]. The amplified DNA was purified using the QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol. Subsequently, the purified DNA fragments were sequenced directly using the ABI Prism Big Dye Terminator V.3.1 Cycle Sequencing Kit on an ABI 3130 DNA automated sequencer (Applied Biosystems). Sequencing reactions were performed in a 20 µl mixture according to the manufacturer's instructions, using the same primers employed for PCR amplification. The newly generated ITS2 sequences were aligned with sequences of species belonging to the family Monorchiidae available on GenBank (**Table 2**) using the basic local alignment search tool (BLAST) [17]. Sequence alignments were conducted using MUSCLE [18] implemented in MEGA10 software [19].

The resulting alignments were refined, and the ends of each fragment were trimmed to match the shortest sequence in each alignment. Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses were employed to determine the relationships of the present isolate and to attempt molecular identification. Maximum Likelihood analysis was performed using MEGA10 software, employing the best-fit model (Kimura 2-parameter), with nodal support estimated using 1,000 bootstrap resamplings. Maximum Parsimony analysis was also conducted using MEGA10 software, with nodal support estimated using 1,000 bootstrap resamplings, and a 50% consensus tree was calculated.

3. Results and Discussion

The present study aimed to characterize and diagnose *P. prominens* Wee, Cribb, and Cutmore, 2022, collected from the intestine of the Red Sea goatfish, *Pa. forsskali* (Fourmanoir and Guézé, 1976) (Family: Mullidae Rafinesque, 1815) in the Red Sea, Egypt. *P. prominens* was isolated from 29 out of 68 fish specimens (17 male and 12 female), accounting for 42.6% of the total sample (51.5% male and 34% female). The mean intensity of infection was 3.7 worms per infected fish. The size and weight of the samples ranged from 14 to 26 cm and 0.31 to 0.173 gm, respectively.

3.1. Morphological Description

The morphology of *P. prominens* is described as follows: The trematode's body is elongated, slightly fusiform, measuring 0.336-1.990 (mean 1.077) mm in length and 0.120-0.488 (mean 0.297) mm in width, with a maximum width in the hindbody. The forebody occupies 39-51% (mean 43%) of the body length, while the hindbody occupies 43-61% (mean 43%) of the body length. The tegument is thin with minute spines distributed throughout the body, and the eye pigment is absent. The oral sucker is subterminal, funnel-shaped, measuring 0.042-0.154 mm in length and 0.047-0.159 mm in width. The prepharynx is very short, while the pharynx is muscular and subspherical, measuring 0.017-0.094 mm in length and 0.019-0.098 mm in width. The esophagus is long, bifurcated in mid-forebody anterior to the ventral sucker into two intestinal caeca. The intestinal caeca are blind, broad, thick-walled, and extend posteriorly in the post-testicular zone. The ventral sucker is round, located in the middle of the body, and measures 0.035-0.110 mm in length and 0.035-0.104 mm in width. The ovary is smooth, oval to triangular, located anterior half of the hind body slightly posterior to the ventral sucker. The uterus is confined to the hind body, with ascending coils forming the metraterm. The terminal organ is sinistro-ventral to the cirrus sac, unipartite, spined, and measures 30-58 mm long by 23-47 mm in width. The eggs are slightly tanned, operculate, unfilamented, and measure 15-28 µm in length and 11-16 µm in width. The vitellarium consists of two lateral fields of large round densely clustered follicles distributed from a short distance posterior to the ventral sucker to the level of the ovary. The excretory vesicle is tubular, intercaecal, extended to the posterior margin of the testis, and terminates at the posterior end of the body. The testis is single, entire, subspherical, located in the middle of the hindbody, and overlaps one or both caeca. The cirrus sac is located in the middle third of the body, sub-cylindrical, median, and intercaecal. The genital atrium lacks spines and is transversely oval. The common genital pore is small, median, and immediately anterior to the ventral sucker. (**Figs. 1 & 2**).

Specimens recovered from *Pa. forsskali* were described as belonging to the genus *Proctotrema* Odhner, 1911, based on the site of infection, body morphology, and tegumental characteristics. The body was small and fusiform, with a spined tegument. The oral sucker was accurate and subterminal, while the ventral sucker was located in the anterior half of the body. The pharynx was small, and the intestinal bifurcation occurred well anterior to the ventral sucker, with blind caeca. The genital opening was submedian and postbifurcal. The cirrus sac was club-shaped, located in the forebody, postbifurcal, and enclosed a large internal seminal vesicle, a well-developed prostatic complex, and an armed cirrus. The ovary was located anteriorly in the hind body, and the terminal organ was saccular, lined with circular spines [14]. Additionally, the testis was single, located in the anterior half of the hindbody, and the uterine coils occupied most of the hindbody. The follicular vitellarium was located posterior to the ventral sucker and anterior to the ovary [5]. These characters collectively placed these specimens in Proctotrema Odhner, 1911. Notably, the species *P. prominens* Wee, Cribb, and Cutmore, 2022 can be distinguished from *Postmonorcheides maclovini* Szidat, 1950 and *Monorcheides popovicii* Szidat, 1950 mainly by having a single testis (versus two testes) and a smooth (versus trilobed) ovary [20]. Moreover, *P. prominens* differs morphologically from other species of *Proctotrema* such as *P. addisoni* Searle, Cutmore, and Cribb,

2014, *P. bacilliovatum* Odhner, 1911, *P. guptai* Ahmad and Dhar, 1987, and *P. elongatum* (Manter, 1931) Wee, Cutmore, Pérez-del-Olmo, and Cribb, 2020, which all possess a uterus described as simply joining the posterior end of the terminal organ without a metraterm [21 -25].

P. amphitruncatum Fischthal and Thomas, 1969, reported from *Pomadasys jubelini* (Family: Haemulidae) from Ghana, can be distinguished from the present specimens by possessing a distinctly elongated body, an oval, thick-walled cirrus sac, a ventral sucker that is distinctly smaller than the oral sucker, and caeca that do not reach the posterior extremity but are much closer to the latter than to the testis [26]. Similarly, *P. bartolii* Carballo, Laurenti, and Cremonte, 2011, reported from *Odontesthes smitti*, *O. nigricans* (Family: Atherinopsidae), and *Eleginops maclovinus* (Family: Eleginopidae) off Patagonia, Argentina, can be distinguished from the present specimens by possessing a round and unspecialized oral sucker, suckers of nearly equivalent size, and caeca well anterior to the posterior body extremity [27]. Additionally, *P. odhneri* Ramadan, 1985, nec Srivastava, 1939, reported from *Rhabdosargus haffara* (Family: Sparidae) off the Red Sea, Egypt, can be distinguished from the present specimens by possessing a bilobed ovary, caeca terminating in the pre-testicular region, and a spined genital atrium [28, 29]. The species previously known from the family Mullidae, *P. bacilliovatum* Odhner, 1911, reported from *Mullus barbatus* and *M. surmuletus* of the Mediterranean Sea, Marseille, differed from the present specimens in possessing a uterus that simply joins the posterior end of the terminal organ without a metraterm and a lobed ovary compared to the unlobed ovary of *P. prominens*.

The comparison between the newly collected specimens and the previously described forms, as shown in (Table 1), indicates that the present specimens were identical to *P. prominens* Wee, Cribb, and Cutmore, 2022, based on shared main characteristics and dimensions of all body parts.

3.2. Molecular Phylogeny

The genotypes of *P. prominens* Wee, Cribb, and Cutmore, 2022 (470 nucleotides) were deposited in GenBank with the accession number OP562704. This sequence was aligned with 8 reference sequences representing all available and appropriate species of the Monorchiidae family: two sequences from *Proctotrema* Odhner, 1911 [30, 21], one sequence from *Retroporomonorchis* Wee, Cribb, Cutmore, and Martin, 2020 [31], one sequence from *Paralasiotocus* Wee, Cutmore, Pérez-del-Olmo, and Cribb, 2020 [25], two sequences from unidentified species of *Postmonorchis* Hopkins, 1941 [32, 33], one sequence from *Parachrisomon* Madhavi, 2008 [34], and one sequence from *Hurleytrematoides* Yamaguti, 1954 [35]. For outgroup comparisons, one sequence representing one species from *Asymphylodora* Looss, 1899 [36] was included (Table 2).

The phylogenetic analysis of this dataset revealed that the ingroup taxa of the Monorchiidae formed a monophyletic clade, excluding the outgroup taxa with significant support (ML=74; MP=63). Both Maximum Likelihood and MaximumParsimony analyses of the ITS2 dataset produced phylogenies with identical topologies. Two distinct clades were identified with multiple congeners: species of *Proctotrema* Odhner, 1911, species of *Paralasiotocus* Wee, Cutmore, Pérez-del-Olmo, and Cribb, 2020, and species of *Postmonorchis* Hopkins, 1941, each forming strongly supported clades. *P. addisoni* formed a distinct clade that was basal to *P. prominens*. *Retroporomonorchis pansho* formed a sister clade with *Paralasiotocus abstrusus* in a highly supported clade (99/95). The two unidentified *Postmonorchis* sp. each formed sister clades with the other in a well-supported clade (100/100) (Figs. 3 & 4).

Fig 1: Photomicrograph of *P. prominens* (Wee, Cribb, and Cutmore, 2022**)**.

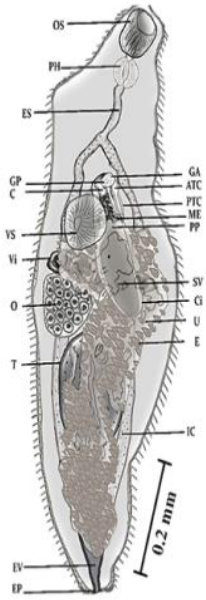

Fig. 2: Camera Lucida Drawing of *P. prominens* (Wee, Cribb, and Cutmore, 2022). (OS) Oral Sucker, (PH) Pharynx, (ES) Esophagus, (GP) Genital Pore, (GA) Genital Atrium, (ATC) Anterior Terminal Chamber, (PTC) Posterior Terminal Chamber, (ME) Metraterm, (PP) Pars Prostatica, (C) Cirrus, (VS) Ventral Sucker, (Vi) Vitellarium, (SV) Seminal Vesicle, (Ci) Cirrus sac, (O) Ovary, (U)Uterus, (E) Egg, (T) Testis, (IC) Intestinal Cecum, (EV) Excretory Vesicle, and (EP) Excretory Pore.

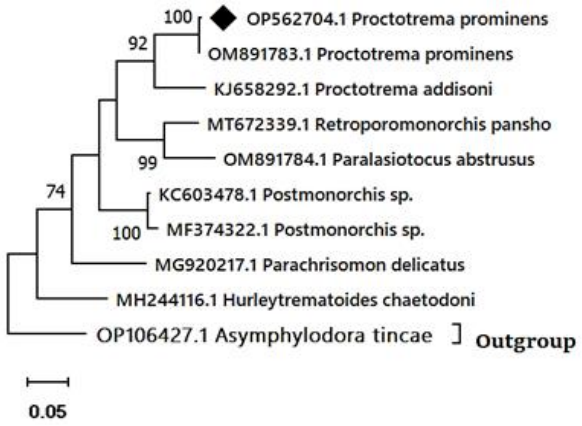

Fig. 3: Genetic Relationships among Members of *P. prominens* (Wee, Cribb, and Cutmore, 2022) Inferred from ITS2 rDNA Locus using Maximum Likelihood Method.

Table 1: Comparison between *P. prominens* (Wee, Cribb, and Cutmore, 2022) of the present specimens and previously described Forms.

Reference	[30]	Present study
Fish host	*Plectorhinchus albovittatus* (Family: Haemulidae)	*Parupeneus forsskali* (Family: Mullidae)
Locality	Queensland, Australia	Safaga, Egypt in the Red Sea
Site of infection	Intestine	Intestine
Body length	590–957 (789) µm	0.336-1.990 (1.077)
Body width	156–218 (184) µm	0.120-0.488 (0.297)
Body length/Body width	3.7-5.5 (4.3)	2.7-4.9 (3.7)
Forebody length	376–414 (357) µm	0.140-0.736 (0.444)
Forebody length as % of body length	40.6–49.2 (44.3) %	39-51 (43) %
Oral sucker length	65–98 (83) µm	0.042-0.154 (0.107)
Oral sucker width	74–106 (89) µm	0.047-0.159 (0.117)
Ventral sucker length	39–82 (59) µm	0.035-0.110 (0.106)
Ventral sucker width	46–79 (63) µm	0.035-0.104 (0.103)
Suckers length %	48.8–86.2 (71.7) %	61–86 (79). %
Suckers width %	50.6–87.5 (70.6) %	53-85 (72.2) %.
Pharynx length	36–50 (44) µm	0.017-0.094 (0.050)
Pharynx length (% of oral sucker length)	45.1-63.1 (53.6) %	47-62 (44) %
Pharynx width (% of oral sucker width)	48.1-61.6 (55.6) %	50-60 (47) %.
Esophagus length	96–165 (128) µm	0.031-0.242 (0.134)
Esophagus length as % of body length	12.9–20.6 (16.0) %	9-20 (13) %
Pre-bifurcal region (% of body length	26.5-30.3 (28.8) %	26.2-30.7 (28.5) %
Caecal termination from the end of body % of body length	1.9-12.9 (6.6) %	5-12 (11.4) %
Testis length	103–183 (140) µm	0.092-0.288 (0.176)
Testis width	63–124 (93) µm	0.043-0.154 (0.110)
Testis to ventral sucker (% of body length)	7.0-17.8 (13.0) %	7.3-17 (14) %
pre-testicular region as % body length	57.8–68.1 (64.2) %	58-66 (61) %
Post-testicular region as % body length	15.2–24.8 (18.9) %	15-23 (22) %
Cirrus sac length	167-348 (232) µm	0.160-0.320 (0.197)
Cirrus sac width	31-72 (45) µm	0.033-0.084 (0.075)
Seminal vesicle length	56–128 (86) µm	0.040-0.132 (0.109)

Table 1 Complimentary:

Reference	[30]	Present study
Cirrus sac width	31-72 (45) µm	0.033-0.084 (0.075)
Seminal vesicle length	56–128 (86) µm	0.040-0.132 (0.109)
Cirrus sac width	31-72 (45) µm	0.033-0.084 (0.075)
Seminal vesicle length	56–128 (86) µm	0.040-0.132 (0.109)
Cirrus sac width	31-72 (45) µm	0.033-0.084 (0.075)
Ovary width	41–64 (52) µm	0.044-0.066 (0.053)
Pre-ovarian zone (% of body length)	49.1-61.8 (56.4) %	49-60 (57.4) %
Post-ovarian zone (% of body length)	30.9-42.9 (35.6) %	30-41.1 (39) %
Terminal organ length	32-59 (43) µm	30-58 (41)
Terminal organ width	21-46 (29) µm	23-47 (30)
Vitellarium range (% of body length)	10.1-15.7 (12.6) %	10-15 (14) %
Eggs length	17–29 (26) µm	15-28 (23) µm
Eggs width	10–16 (13) µm	11-16 (14) µm

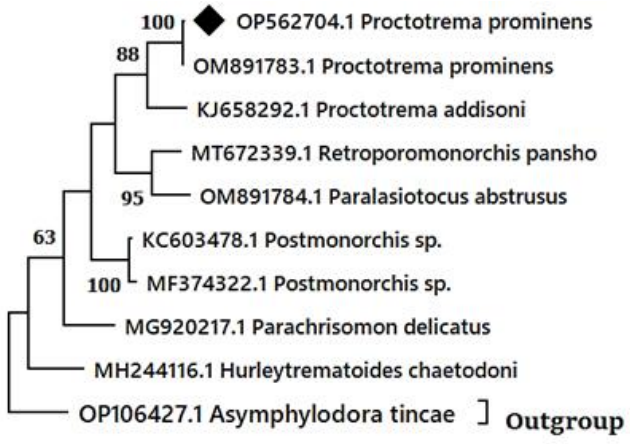

Fig. 4: Genetic Relationships among Members of *P. prominens* (Wee, Cribb, and Cutmore, 2022) Inferred from ITS2 rDNA Locus using Maximum Parsimony Method.

The phylogenetic relationship studied using maximum likelihood and maximum parsimony methods showed that the present specimen is more closely related to *P. prominens*. The sequence data of ITS2 rDNA for the present trematode demonstrated similarity with other Monorchiinae species and supported its taxonomic position with the genus *Proctotrema* with a close relationship with *P. prominens* as a more related sister taxon. The comparative sequence analyses (**Table 2**) demonstrated that the IST2 rDNA sequence of the present isolated from the *Pa. forsskali* identified as *P. prominens* was identical to the sequence of the same species reported in *Plectorhinchus albovittatus*.

Table 2: presents sequence data representing various species of the Monorchiidae family, along with an outgroup taxon, determined in the present study. The table includes information on the taxon, host species, locality, and GenBank accession numbers for each sequence.

Taxon	Host	Locality	*GenBank accession Nos.
Proctotrema prominens	*Parupeneus forsskali*	Red Sea, Egypt	OP562704
P. prominens	*Plectorhinchus albovittatus*	Australia	OM891783
P. addisoni	*Diagramma labiosum*	Australia	KJ658292
Postmonorchis sp.	*Donax trunculus*	Italy	KC603478
Parachrisomon delicatus	*Upeneus tragula*	Australia	MG920217
Retroporomonorchis pansho	*Lutjanus fulvus*	Australia	MT672339
Hurleytrematoides chaetodoni	*Chaetodon striatus*	USA	MH244116
Postmonorchis sp.	*Ostrea edulis*	Italy	MF374322
Paralasiotocus abstrusus	*Plectorhinchus albovittatus*	Australia	OM891784
	Outgroup:		
Asymphylodora tincae	*Tinca tinca*	Lithuania:	OP106427

***GenBank Accession Nos.**: Lists the GenBank accession numbers assigned to the sequences, allowing researchers to access and reference the genetic data.

The redescribing of *P. prominens* from a new fish host record holds several significant implications in the field of parasitology and fish biology. Here are some key points highlighting the significance of this study:

1. **Taxonomic Confirmation: By redescribing P. prominens,** the study provides a detailed and updated taxonomic description of the trematode parasite. This contributes to the accurate identification and classification of the species, improving our understanding of its morphological characteristics and life cycle.
2. **Host-Parasite Relationship:** The identification of *P. prominens* in a new fish host, *Pa. forsskali*, expands our knowledge of the host-parasite relationship. It confirms the ability of *P. prominens* to parasitize a broader range of fish species and provides insights into its host specificity and ecological interactions.
3. **Geographic Distribution**: The study reports the presence of *P. prominens* in the Red Sea at Safaga, Egypt, which adds to our understanding of the geographic distribution of the parasite. This information is crucial for mapping the distribution patterns of parasitic organisms and studying factors influencing their prevalence and abundance in different regions.
4. **Molecular Identification**: The use of molecular techniques, specifically IST2 rDNA sequencing and phylogenetic analysis, enhances the accuracy of species identification. By comparing the IST2 sequence with existing data in GenBank, the study confirms the taxonomic placement of *P. prominens* within the monorchiid group, providing valuable genetic information for future studies on related species.
5. **Host-Parasite Dynamics**: The discovery of *P. prominens* in *Pa. forsskali* contributes to our understanding of the host-parasite dynamics within the Red Sea ecosystem. It sheds light on the susceptibility of specific fish species to parasitic infections and provides insights into the potential impacts of the parasite on the health and ecology of the host population.

4. Conclusion

This study presents the first redescription of the trematode parasite *P. prominens* from a new fish host record, *Pa. forsskali*, from the Red Sea at Safaga, Egypt, representing a new locality. Additionally, the study includes phylogenetic analysis. Overall, redescribing *P. prominens* from a new fish host record expands our knowledge of the species taxonomy, host range, geographic distribution, and molecular characteristics. This information is essential for advancing our understanding of parasite-host interactions, ecosystem dynamics, and the broader field of parasitology.

Credit authorship and contribution statement:

M.E. and Z.A.; methodology, X.X.; software, R.K.; validation, R.K. and T.H.; formal analysis, M.E. and Z. A.; investigation, Z.A.; resources, M. E. and Z.A.; data curation, M.E. and Z.A.; writing—original draft preparation, T.H.; writing review and editing, R.K. and T. H.; visualization, R. K., T.H., and M. E.; supervision, M. E. and Z.A.; project administration, M.E. and Z.A.; funding acquisition, Sohag University. All authors have read and agreed to the published version of the manuscript."

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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