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Hysterothylacium Persicum (Nematoda: Raphidascarididae) Parasite of Orangespotted Grouper *Epinephelus coioides* (Forsskål, 1775) Iraqi Marine Water Fishes

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Abstract

Hysterothylacium is one of the most important nematode parasites parasitizing fish, and it's the most diverse species nematodes of marine parasites, these species attach to the intestinal wall via their mouth lips adaptation. In the present study, seven out of 56 fish specimens (12.5 %) were found to be infected by the adult worms of Greasy grouper fishes **Orangespotted grouper *Epinephelus coioides* (Forsskål, 1775)**from locations of marine coastal water, Arabian Gulf, Iraq. A new recorded of *Hysterothylacium* spp. collected are morphologically described, genetically and Sccaning Electron Microscope (SEM) images for new systematic observations, on dorsal labium, sub ventral labia, posterior end of a the female and the male. Based on the study of the gene sequencer data and SEM images, it will be suggested that species are *H. persicum.* The study provides us some data on the taxonomy of these parasites in the Arabian Gulf. The gross examination of the fish's intestine food items and the Relationships of fish length, In this study showing that the length ranges for adult infected were 25-45 cm long, and the major food items found in the stomach were a Largest group of miscellaneous of fishes length more than 25 cm , whereas crustaceans miscellaneous parts of animal body, i.e. appendages, chela, parts of the carapace of crustaceans were found in stomachs in all the month including except some of them in stomach of length less than 20 cm , it was clear that the 25-45cm ctotal length feed mainly on fish that explain fishes are the second intermediate host of IV larval stage of nematodes. In the present reported can consider that the *Epinephelus **coioides*** as a new host for *Hysterothylacium persicum* and a newly recorded in Iraqi marine water . The aim of the current study is to diagnose and study new species of parasites that belong to the genus throughout the morphological studies along with the image data selected for SEM and sequence and combined with the other species of *Hysterothylacium* that reside in the Iraqi marine water and the relationships of fish length, food items and parasite infection.

Keywords: *Hysterothylacium persicum,* Nematoda,**Orangespotted grouper *Epinephelus coioides (Forsskål, 1775)*** fish, Iraqi marine waters.

Hysterothylacium persicum (Nematoda: Raphidascarididae) الطفيلي الخيطي Orangespotted grouper *Epinephelus coioides* المصيب لاسماك الهامور (Forsskål, 1775) في المياة البحرية العراقية

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الخلاصة

الطفيلي الخيطي ***Hysterothylacium*** يعد احد أهم طفيليات الأسماك، وتعد الأنواع البحرية الأكثر تنوعاً وشيوعا ، هذه الأنواع تلتصق بجدار الأمعاء عن طريق التكيف مع شفاه الفم. وجد من خلال الدراسة الحالية ان سبعة اسماك كانت مصابة من اصل ٥٦ سمكة تم فحصها بنسبة اصابة (١٢.٥ %) بالديدان في اسماك الهامور ***Orangespotted grouper Epinephelus coioides (Forsskål, 1775)*** ، من مواقع مختلفة من الاسماك المجمعة من منطقة بيع الاسماك في مدينة الفاو والمصطاده من المياه الساحلية البحرية العراقية من الخليج العربي. اجريت على النوع الجديد المعزول في الدراسة الحالية الدراسات الوصفية والجينيه وصور بالمجهر الالكتروني لتراكيب الشفاه ونهاية الطفيلي . استناداً إلى دراسة البيانات التسلسل الجيني وصور المجهر اللاكتروني ، يعد النوع المسجل في الدراسة الحالية هو ***H. persicum***. حيث قدمت الدراسة لنا بعض البيانات حول تصنيف هذه الطفيليات في الخليج العربي. اجريت دراسة حول غذاء الاسماك ومحتويات الأمعاء وعلاقاتها مع اطوال الأسماك والاصابة ، تبينان اطوال الاسماك المصابة تروح بين مجموعة طول ٢٥-٤٥ سم، والعناصر الغذائية الرئيسية الموجودة في المعدة كانت مجموعة متنوعة من الاسماك ، بينما شكلت اجزاء من الجسم لمجموعة من القشريات في جميع اشهر الجمع بما في ذلك ما عدا بعض منها في المعدة لمدة أقل من ٢٠ سم، أنه وكان واضحا أن الطول الإجمالي ٢٥-٤٥ سم تتغذى أساسا على الأسماك التي تشرح الأسماك هي المضيفة الوسيطة الثانية من المرحلة الرابعة اليرقات من الديدان الخيطية. نتائج الدراسة الحالية عدت اسماك الهامور هي مضائف جديده لهذه النوع من الطفيليات والنوع المسجل في الدراسة الحالية هو تسجيل جديد في المياه البحرية العراقية . هدف الدراسة الحالية هو تشخيص ودراسة أنواع جديدة من الطفيليات التي تنتمي إلى جنس ***Hysterothylacium*** اعتمادا على الدراسات المورفولوجية المعززه بصور المجهر اللالكتروني مع نتائج الدراسات الجينية ومقارنتها مع الأنواع الأخرى من النوع المسجلة في المياه البحرية العراقية ودراسة علاقة بين طول الأسماك والغذاء والإصابة بالطفيليات

Introduction

Greasy grouper fish, Orangespotted grouper *Epinephelus coioides (Forsskål, 1775)*wild fish are distributed worldwide, Indo-Pacific, Red Sea to South Africa, Northern Japan, and southern New South Wales and Lord Howe Island, the adults of fish species' habits are in the areas of coral reefs, found in deeper waters, and exclusively feed on fishes (holocentrids, mullid, and pomacentrid); crustaceans, the juveniles had taken from coral reefs and flats in tidal pools[1].

The family Anisakidae has been Universal distributed, it is found in the marine teleosts in temperate and cold [2], and few has been recording freshwater fish [3]. *Hysterothylacium* parasite species are mainly in the marine fish parasites, and their complete life cycle in low on the food chain of some fishes as intermediate host, whereas the large predatory fish species are definitive hosts [4]. Our knowledge of their parasites taxa is a little off over that 300 of fish species are recorded in Iraqi marine waters [5]. There are some reports on the presence of the *Hysterothylacium* species in Iraqi marine water fishes, most of the study is on the morphology, so that providing us limited taxonomical information. The aim of the current study is to diagnose and study new species of parasites that belong to the genus throughout the morphological studies along with the image data selected for SEM and sequence and combined with the other species of *Hysterothylacium* that reside in the marine water area **.**

Materials and methods

1- Fish collection and examination of nematodes

Fishes were purchased at the local fish market in Al- Faw City from the locations of the Khor Adulah area in Southern Iraq, Arabian Gulf (29°58 0 33 00 N48°28 0 20 E) during the years 2017 Figure-1. The intestine of 56 fish specimens of **Orangespotted grouper *Epinephelus coioides* (Forsskål, 1775)** was examined. Fish collection transferred to the Parasite Laboratory, Marine Science Center, University of Basra, Iraq.. The nematode was cleared in lactophenol for morphological examination. Fish divided as a total height and not depending on the sex in order to study the relationship infection with length, some fish injected with formalin 10% concentration to maintain food digestion in order to study the relationship with food items. Nematodes were identified using the

morphology of the labia, the position of the excretory pore, the esophageal ventriculus, ventricular appendix and the tail; molecular sequencer and SEM Image. All measurements are given in millimeters. Fish identified According Fishbase [1].

Samples of parasites and fish tissue that had been fixed and stored in 70% ethanol are sent to Dr. Shamasi, Charles Sturt University, Australia for the molecular examination,

2- Molecular analysis

The polymerase chain reaction (PCR) were processed following standard methods are described by Shamsi *et al.*, [6-8] these included critical point (Molecular analysis Genomic DNA was isolated from individual by sodium dodecyl sulphate/proteinase K treatment, column-purified (Wizard™ DNA Clean-Up, Promega, Madison, Wisconsin, USA) and eluted into 40 µl of water , host DNA was isolated from the musculature of fish using the same method. The poly- merase chain reaction (PCR) was used to amplify the ITS-1 and ITS-2 regions using primers and cycling conditions. Samples with fish DNA or without genomic DNA were included in the PCRs as negative controls; no amplicons were produced in the PCR from these samples. An aliquot (4 µl) of each amplicon was examined on a 1.5% w/v agarose gel, stained with ethidium bromide and photographed using a gel documentation system. Amplicons were purified over mini-columns (Wizard™ PCR Prep, Promega), eluted in 30 µl of water and then were sent to Australian Genomic Research Facilities to be subjected to Sanger sequencing, in both directions, using the same primers as for PCR. Sequences were aligned using the computer program Clustal and then adjusted manually. Polymorphic sites were designated using International Union of Pure and Applied Chemistry (IUPAC) guidelines.)

3- Scanning Electron Microscope (SEM)

Some parasites fixed and stored in 70% ethanol were processed following standard methods for SEM. Scanning Electron Microscope with digital images was obtained with the Nano lab software Images were taken with the help of Dr Omar M. Amin and his group of Electron Optics Laboratory (BYU) in Institute of Parasitic of Diseases, Scottsdale, USA Institutional Parasitology for SEM image these included critical point (Samples of parasites that had been fixed and stored in 70% ethanol were processed following standard methods. These included critical point drying (CPD) in sample baskets and mounting of SEM sample mounts (stubs) using conductive double sided carbon tape. Samples were coated with gold and palladium for 3 minutes using a Polaron #3500 sputter coater (Quorum (Q150 TES) www.quorumtech.com) establishing an approximate thickness of 20 nm. Samples were placed and observed in an FEI Helios Dual Beam Nanolab 600 (FEI, Hillsboro, Oregon) Scanning Electron Microscope with digital images obtained with the Nano lab software system (FEI, Hillsboro, Oregon). Images were taken at various magnifications. Samples were received under low vacuum conditions using 10 KV, spot size 2, 0.7 Torr using a GSE detector).

Figure 1-photograph showing study area Iraqi marine coastal water, Arabian Gulf. (29°58,33, N48°28, 20E).

Result and Discussion

Hysterothylaciu persicum Figures- 2, 3, and 4

Males: Body length 30.5 (22.0–39.0) width 0.67 (0.47 – 0. 87). The mouth have 3 labia, one dorsal measured 0.23 (0.17–0.29) long, 0.2 (0.15–0.26) wide, and two are subventral labium 0.19 (0.12–0.27) long, 0.09 (0.06– 0.13) wide. Nerve ring was 0.48 (0.49– 0.92). Excretory pore below the nerve ring from anterior end.

Females: Body length is 14.9 (7.3–22.5), width 0.49 (0.18–0.8). Mouth has also had three labia, one dorsal labium 0.12 (0.04–0.2) long, 0.06 (0.02–0.11) wide, and two subventral labia 0.1 (0.03–0.17) long, 0.095 (0.01–0.18) wide. Nerve ring was 0.53 (0.25–1.01) from the anterior end. Excretory pore near the nerve ring, 0.9 (0.3– 1.5) from anterior end of the body, all measurement are in the mm.

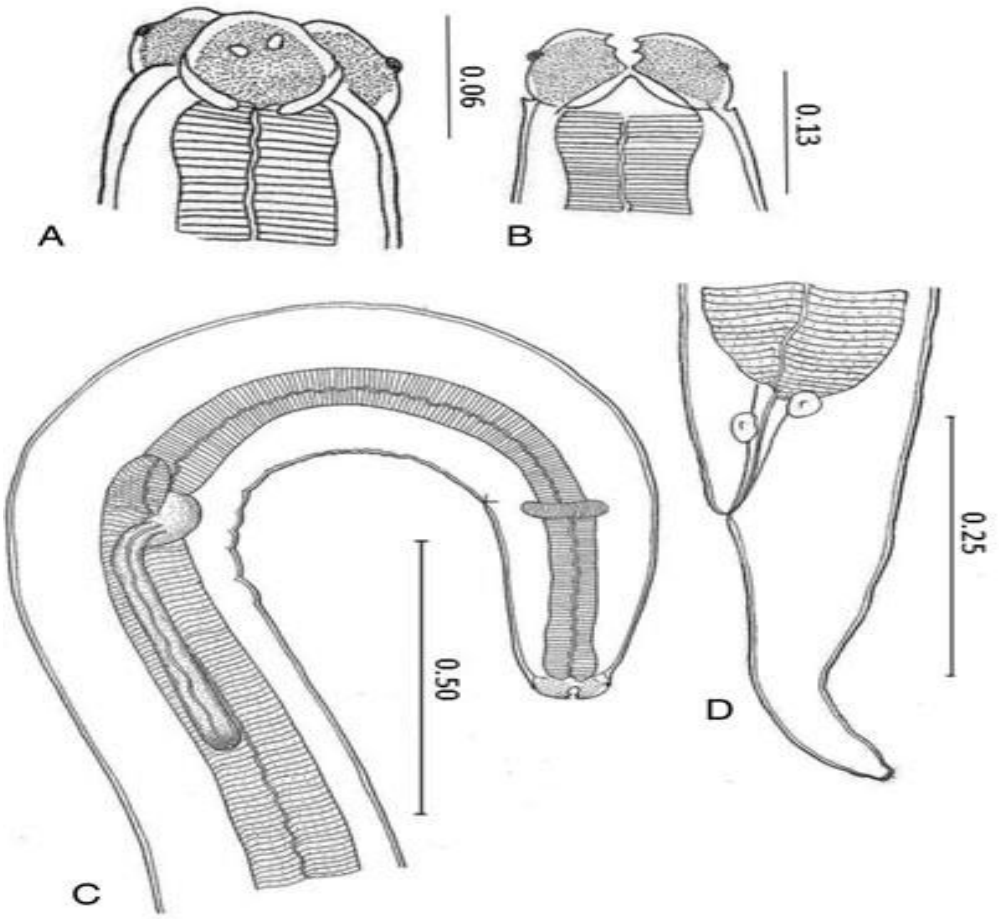

Figure 2-*Hysterothylacium persicum*. (A) Dorsal labium, (B) subventral labia, (C) posterior end of male (D) anterior end of *Hysterothylacium* spp. adults. Scale bars are given in mm.(refrance . Ghadam et al., 2018)[9].

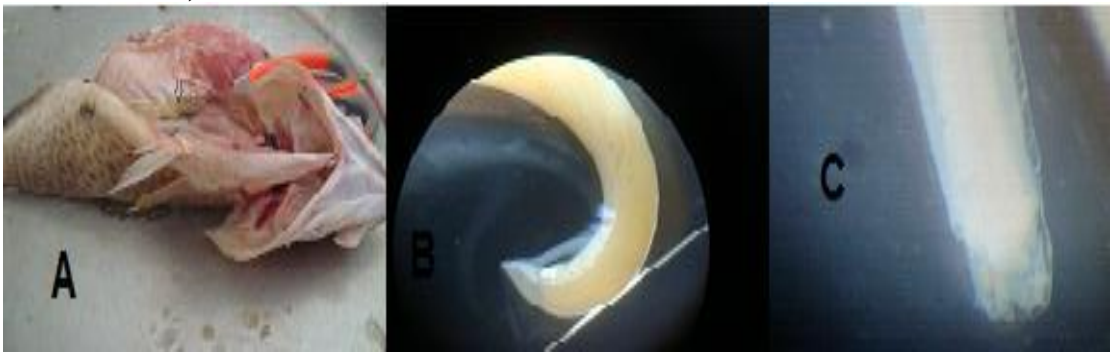

Figure 3-(A: Greasy grouper fishes, *Epinephelus tauvina* ; B posterior end of male ; C anterior end of male.

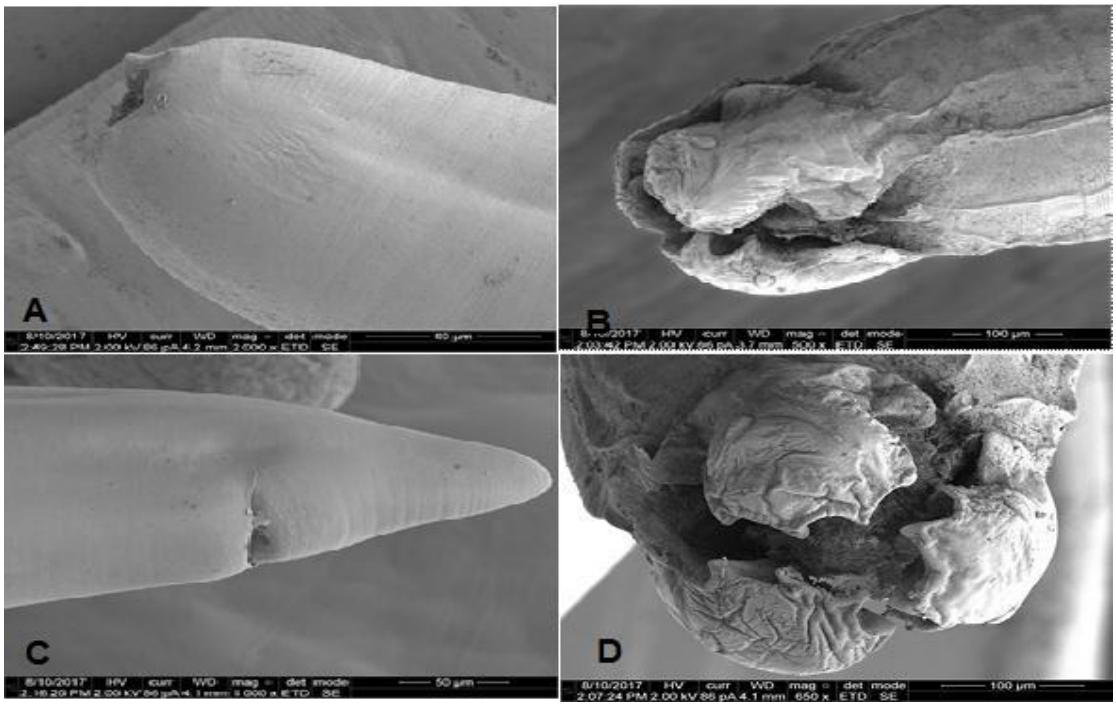

Figure 4-Scanning electron micrographs of *Hysterothylacium persicum* from Greasy grouper fishes, *Epinephelus tauvina* from Iraqi marine waters (B) Anterior region of the body, sublateral view (lateral and excretory pore arrowed). (A, C) Posterior end of body, lateral view (paracloacal and postcloacal papillae arrowed) (D) Cephalic extremity, apical view.

The molecular data of the description of specimens are compared with those deposited in GenBank in Australia and the result shows that they are identical in (99 %) with adult *H. persicum* (Gen Bank accession numbers that registered by [9, 10] .

Fish length, Food Items and parasite infection relation.

The relationships of fish length, Food items and parasite infection, showing that total of 56 specimens of geasy grouper fishes, Orangespotted grouper *Epinephelus coioides coioides* (Forsskål, 1775) were examined, the major food items found in the stomach are a largest group of miscellaneous of fishes length more than 25 cm , whereas crustaceans miscellaneous parts of animal body, i.e. appendages, chela, parts of carapace of crustaceans were found in stomachs in all the month including except some of them in stomach of length less than 20 cm. Foods in relation to size of fishes are occurrence of various food elements were distinctly different as related to the fish size, it was clear that the 25-45 total length feed mainly on fishes, most of fishes in this total rang lengh are infected with unidentified larval stage and the IV larval stage of nematodes.

According to Li *et al.* [11] and Li *et al.* [12] the genus *Hysterothylacium* Ward & Magath, 1917, include 70 valid species, most of them are parasitic on marine, estuarine, and freshwater fishes, except *Hysterothylacium burtti* Raffel & Anderson, 2009, found in amphibians [13]. *H. persicum* was first described in the Arabian Gulf by Shamsi *et al*. [10]. The cmparison collected of morphologically described, and some of genetically and SEM images for new observations, on the structure of the labium, of a female and male among *the geneus Hysterothylacium* and our specimens in the present study and those in the previous studies does not show a significant difference just in the size of parasite total length. The result of presnt study are agree with suggested of Zhao *et al*.[14] and Mattiucci and Nascetti [15] identified the existence of "sibling species" within the ascaridoids, morphologically very similar but genetically different in some speciemens because of a having distinct host preferences, life cycles and geographical distribution, for that reason the species need more specific study about the gnetic variation and its relationship to the environment change and host gene.

In the present study report Orangespotted grouper *Epinephelus coioides* (Forsskål, 1775) as consider a new host for *Hysterothylacium persicum*.and the parasite a new record in Iraqi marine

water. In conclusion, the present study showed the presence of fourth members of the genus *Hysterothylacium* in Iraqi fish.

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References

1. Fishbase (http://www.fishbase.org/summary/epinephelus-tauvina.html) Froese, R. & Pauly, D. **2016.** FishBase. World Wide Web electronic publication version (10/2016), **39**: 515-529.
2. Berland, B. **1991.** *Hysterothylacium aduncum* (Nematoda) in fish. ICES Identification leaflets for diseases and parasites of fish and shellfish, Leaflet No. **44**, 4 p.
3. Moravec, F. **1994.** *Parasitic nematode of freshwater fishes of Europe. Academia*, Praha, 473
4. Deardorff, T.L. and Overstreet, R.M. **1981.** *Review of Hysterothylacium and Iheringascaris (both previously= Thynnascaris) (Nematoda: Anisakidae) from the nor- thern Gulf of Mexico*. Proceedings of the Biological Society of Washington 93,
5. Froese, R. and Pauly, D. **2016.** FishBase. World Wide Web electronic publication version (10/2016). Available at www.fishbase.org (accessed 10 November 2016).
6. Shamsi, S., Gasser, R., Beveridge, I. and Shabani, A.A. **2008** .Contracaecum pyripapillatum n. sp. and a description of C. multipapillatum (von Drasche, 1882) from the Australian pelican, Pelecanus conspicillatus. *Parasitology Research* ,**103**: 1031–1039.
7. Shamsi, S., Norman, R., Gasser, R. and Beveridge, I. **2009.** Genetic and morphological evidences for the existence of sibling species within *Contracaecum rudolphii* (Hartwich, 1964) (Nematoda: Anisakidae) in Australia. *Parasitology Research*, **105**: 529–538.
8. Shamsi, S., Gasser, R. and Beveridge, I. **2011.** Mutation scanning-coupled sequencing of nuclear ribosomal DNA spacers (as a taxonomic tool) for the specific identification of different *Contracaecum* (Nematoda: Anisakidae) larval types. *Molecular and Cellular Probes*, **25**: 13–18.
9. Ghadam, M., Banaii, M., Mohammed, E.T., Suthar, J. and Shamsi, S. 2018. Morphological and molecular characterization of selected species of Hysterothylacium (Nematoda: Raphidascarididae) from marine fish in Iraqi waters. Journal of Helminthology, Page 1 of 9 © Cambridge University. 1035–1079.
10. Shamsi, S., Ghadam, M., Suthar, J., Ebrahimzadeh Mousavi, H., Soltani, M. and Mirzargar, S. **2016.** Occurrence of Ascaridoid nematodes in selected edible fish from the Persian Gulf and description of *Hysterothylacium* larval type XV and Hysterothylacium persicum n. sp. (Nematoda: Raphidascarididae). *International Journal of Food Microbiology*, **236**: 65–73.
11. Li, L., Liu, Y-Y., Liu, B-C. and Zhang, L-P. **2012.** Morphological and molecular evidence for a new species of the genus Raphidascaris (Nematoda: Anisakidae) from marine fishes from the South China Sea. *Parasitol Res*, **110**: 1473–1479
12. Li, L., Zhang, L.P. and Liu, Y.Y. **2013.** *Hysterothylacium simile* n. sp. and *H. aduncum* (Rudolphi, 1802)(Nematoda: Raphidascarididae) from marine fishes in the Bohai and Yellow Sea, China, with comments on the record of *H. paralichthydis* (Yamaguti, 1941) from Chinese waters. *Syst. Parasitol*. **84**: 57–69.
13. Raffel, TR. and Anderson TK **2009.** A new species of *Hysterothylacium* (Nematoda: Anisakidae) from the stomach of the red-spotted newt, Notophthalmus viridescens, from Pennsylvania fishless ponds. *J Parasitol*, **95**: 1503–1506
14. Zhao, J.-Y., Zhao, W.-T., Ali, A.H. , Chen, H.-X. and Li, L. **2017.** Morphological variability, ultrastructure and molecular characterization of *Hysterothylacium reliquens*. *Parasitology International*, **66**: 831–838.
15. Mattiucci, S. and Nascetti, G. **2008.** Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host parasite coevolutionary processes. *Adv Parasitol*, **66**: 47–148.