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Morphological and molecular characterization of selected species of *Hysterothylacium* (Nematoda: Raphidascarididae) from marine fish in Iraqi waters

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Abstract

Hysterothylacium species are perhaps the most abundant and diverse group of marine ascaridoids; however, their life cycle and specific identification in larval stages in many parts of the world, particularly in Iraqi marine waters, have not been completely understood. In this study three members of the genus *Hysterothylacium* collected from Khor Abdulla in Iraq are morphologically described, genetically characterized and their relationship with other closely related taxa are compared and discussed. A new *Hysterothylacium* larval type in the fourth stage of development is described, and morphological and molecular evidence (based on the sequences of internal transcribed spacers) are provided for its distinction from previously known fourth-stage *Hysterothylacium* larval types. Based on the sequence data it is suggested that the new larval type, which herein was assigned as *Hysterothylacium* larval type XVI, is *H. persicum* which was previously reported from the close proximity in Bandar Abbas, Iran. In addition, two other taxa, including *Hysterothylacium* larval type XV and *H. reliquens*, have been found in the present study, for which new hosts are reported. This study provides some insights into the taxonomy and systematics of these parasites, not only in this region but also for similar studies elsewhere.

Introduction

Hysterothylacium species are marine ascaridoids that complete their life cycle in various fish species. Usually fish low on the food chain act as intermediate/paratenic hosts for *Hysterothylacium* species, whereas large predatory fish are their definitive hosts (Deardorff & Overstreet, 1981). Members of the *Hysterothylacium* species are considered to be of zoonotic significance following a case of human infection with a female *Hysterothylacium aduncum* in Japan (Yagi *et al.*, 1996).

According to Froese & Pauly (2016) over 200 species of fish are found in Iraqi marine waters, many of which are edible; however, our knowledge of their parasites is poor. Although there have been some reports on the presence of

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Hysterothylacium in Iraqi marine fish, most of these are based on morphology only, providing limited morphological description that makes specific identification difficult (Ali *et al*., 2014). Therefore the aim of the present study was to provide a detailed morphology combined with sequence data of the first and second transcribed spacers of the ribosomal DNA (ITS-1 and ITS-2, respectively) for selected *Hysterothylacium* species found in the region.

Materials and methods

Collection and examination of nematodes

Nematode parasites were collected from five species of fish – the areolate grouper, *Epinephelus areolatus* (Forsskål, 1775), tigertooth croaker, *Otolithes ruber* (Bloch & Schneider, 1801), largetooth flounder, *Pseudorhombus arsius* (Hamilton, 1822), brushtooth lizardfish, *Saurida undosquamis* (Richardson, 1848) and oriental sole, *Brachirus orientalis* (Bloch & Schneider, 1801) – from Khor Abdulla, located in the south of Iraq. All fish were examined visually; nematode parasites were collected and preserved in 70% ethanol, and transferred to the Parasitology Laboratory, Charles Sturt University, Australia, where they were prepared for morphological and molecular examination. A small piece of the mid-body of each nematode was excised for molecular study, and the rest of the nematode was cleared in lactophenol for morphological examination, as described previously (Shamsi *et al*., 2008, 2009a, b, 2011). Nematodes were identified using the morphology of the labia, the position of the excretory pore, the oesophageal ventriculus, ventricular appendix and the tail (Shamsi *et al*., 2013, 2015, 2016; Shamsi, 2016). All measurements are given in millimetres as the mean followed by the range in parentheses. The specimens have been deposited in the South Australian Museum, Adelaide (SAM).

Molecular analysis

Genomic DNA was isolated from individual larvae by sodium dodecyl sulphate/proteinase K treatment, column-purified (Wizard™ DNA Clean-Up, Promega, Madison, Wisconsin, USA) and eluted into 40 µl of water (Shamsi *et al*., 2008). Host DNA was isolated from the musculature of fish using the same method. The polymerase chain reaction (PCR) was used to amplify the ITS-1 and ITS-2 regions using primers and cycling conditions described previously (Shamsi *et al*., 2008, 2009a, b, 2011). 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 ClustalX (Thompson *et al*., 1997) and then adjusted manually. Polymorphic sites were designated using International Union of Pure and Applied Chemistry (IUPAC) guidelines.

Results

Three distinct *Hysterothylacium* taxa were found in the examined fish in the present study. Their morphological description and molecular characterization based on the ITS sequence data are provided below and details of the specimens examined in the present study are shown in table 1.

Hysterothylacium *larval type XVI*

Material examined

Ten specimens: specimen number 15-1 from the stomach of *E. areolatus*, museum accession number AHC47861; specimen numbers 2-3, 2-4, 2-10, 2-13, 2-14, 2-15 and 2-16 from the alimentary tract of *P. arsius*, museum accession number AHC47862; and specimen numbers 6-3 and 6-18 collected from the intestine of *S. undosquamis*, museum accession number AHC47863.

Description

Fourth-stage larvae (fig. 1A–D). Body length 7.62 (3.55–10.73; $n = 10$), width 0.21 (0.13–0.35; $n = 10$). Three labia, one dorsal, two subventrals; dorsal labium 0.05 (0.04–0.07; $n = 7$) long, 0.05 (0.03–0.07; $n = 7$) wide, subventral labia 0.05 (0.03–0.08; $n = 8$) long, 0.04 (0.03–0.06; $n = 8$) wide, interlabia 0.02 (0.01–0.02; $n = 8$) long. Nerve ring 0.28 (0.24–0.35; $n = 5$) from anterior end. Excretory pore below the nerve ring, 0.32 (0.28–0.40; $n = 4$) from anterior end. Muscular oesophagus 0.77 (0.58–0.96; $n = 7$) long, 0.09 (0.09–0.10; $n = 7$) of body length. Ventriculus relatively round, 0.08 (0.07–0.09; $n = 2$) long. Ventricular appendix 0.39 (0.34–0.46; $n = 3$) long, 0.47 (0.43–0.50; $n = 3$) of oesophageal length. Intestinal caecum 0.18 (0.16–0.22; $n = 3$) long, 0.22 (0.17–0.26; $n = 3$) of oesophageal length

Table 1. Details of the museum and GenBank accession numbers of the *Hysterothylacium* spp. examined in the present study.

Parasite	Hosts	Museum accession numbers	ITS-1 & ITS-2 (bp), respectively	GenBank accession numbers
Hysterothylacium larval type XVI (L4)	*Epinephelus areolatus*, *Pseudorhombus arsius*, *Saurida undosquamis*	AHC47861–3	436 & 276	LT717074–7
Hysterothylacium larval type XV (L3)	*Otolithes ruber*, *Pseudorhombus arsius*, *Saurida undosquamis*, *Brachirus orientalis*	AHC47864–7	440 & 297	LT717078–9
H. reliquens (adult)	*Otolithes ruber*, *Brachirus orientalis*	AHC47868–9	432 & 341	LT717080–5

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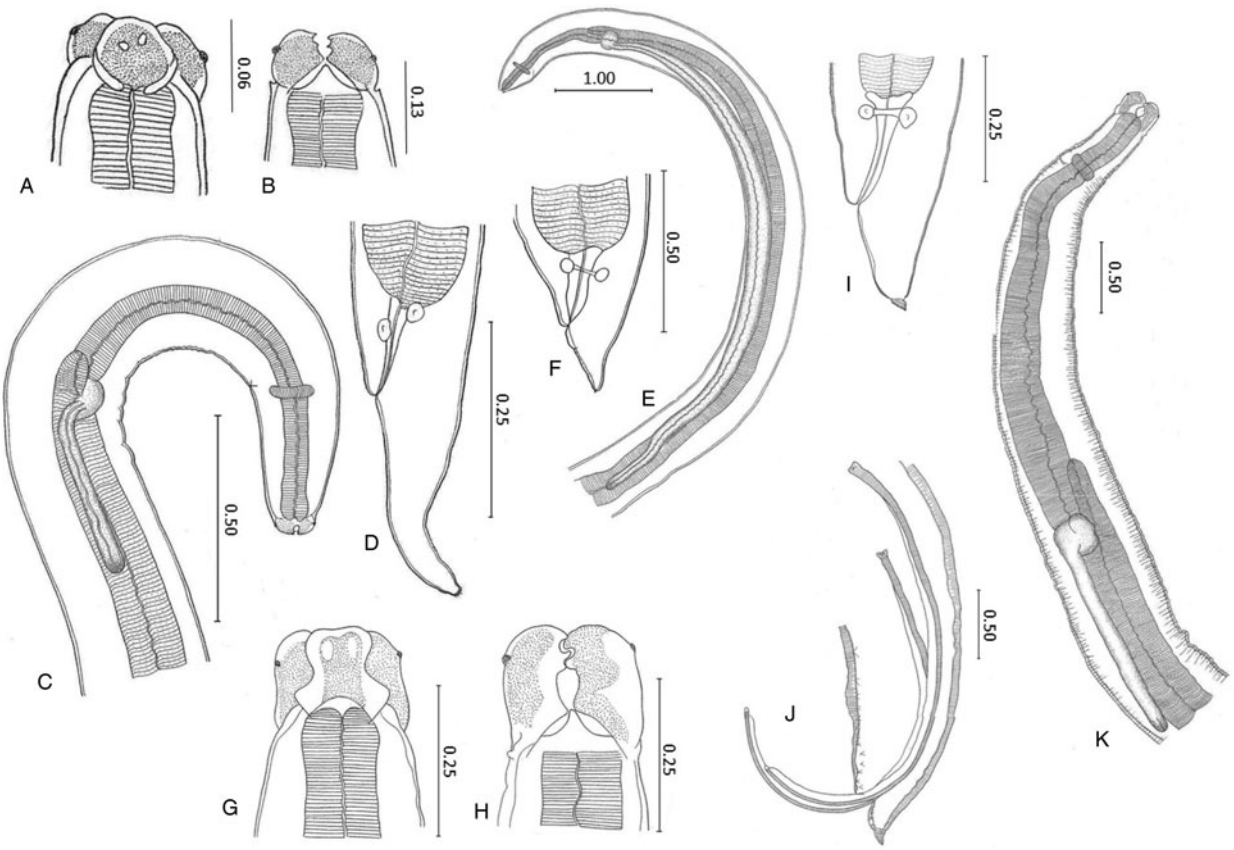

Fig. 1. Larvae of third and fourth stages (L3, L4) and adults of *Hysterothylacium* spp. (A) Dorsal labium, (B) subventral labia, (C) anterior and (D) posterior ends of the *Hysterothylacium* type XVI fourth-stage larva. (E) Anterior and (F) posterior ends of the *Hysterothylacium* type XV third-stage larva. (G) Dorsal labium, (H) subventral labia, (I) posterior end of a female, (J) posterior end of male and (K) anterior end of *Hysterothylacium reliquens* adults. Scale bars are given in millimetres.

and 0.48 (0.35–0.59; $n = 3$) of ventricular appendix length. Tail 0.21 (0.16–0.30; $n = 8$) long, 0.09 (0.05–0.14; $n = 8$) wide, 0.03 (0.02–0.04; $n = 8$) of body length, with relatively few sharp spines at tip.

Molecular characterization

ITS-1 and ITS-2 were 436 and 276 bp long, respectively (GenBank accession numbers: LT717074–7). They were, respectively, 99.5% and 100% identical to *Hysterothylacium persicum* (GenBank accession numbers LT576366, LT576367, LT576368 (ITS-1) and LT576369, LT576370, LT576371 (ITS-2)).

Hysterothylacium *larval type XV of Shamsi, Ghadam, Suthar, Ebrahimzadeh Mousavi, Soltani, & Mirzargar, 2016*

Material examined

Twenty-two specimens: specimen numbers 20-16 and 20-19 collected from the intestine of *O. ruber*, museum accession number AHC47864; specimen numbers 2-5, 2-6, 2-21, 2-23 and 2-24 collected from the intestine of *P. arsius*, museum accession number AHC47865; specimen numbers 6-4, 6-6, 6-10, 6-11 and 6-16 collected from the intestine of *S. undosquamis*, museum accession number AHC47866; and specimen numbers 1-1, 1-9, 1-19, 1-21, 1-23, 1-25, 1-26, 1-29, 1-36 and 1-38 collected from the liver of *B. orientalis*, museum accession number AHC47867.

Description

Third-stage larvae (fig. 1E and F), labia not developed. Body length 13.86 (5.88–20.95; $n = 22$), width 0.52 (0.25–0.68; $n = 22$). Tooth present. Nerve ring 0.26 (0.16–0.35; $n = 17$) from anterior end. Excretory pore below the nerve ring, 0.31 (0.17–0.42; $n = 16$) from anterior end. Oesophagus 1.00 (0.58–1.42; $n = 22$) long, 0.07 (0.06–0.10; $n = 22$) of body length. Ventriculus relatively round, 0.11 (0.08–0.14; $n = 19$) long. Ventricular appendix 4.43 (1.92–6.53; $n = 20$) long, 4.35 (3.14–5.46; $n = 20$) times oesophageal length. Intestinal caecum short, 0.14 (0.08–0.21; $n = 14$) long, 0.15 (0.06–0.36; $n = 14$) of oesophageal length and 0.03 (0.02–0.05; $n = 13$) of ventricular appendix length. Tail pointed with a nodular protuberance, 0.17 (0.13–0.22; $n = 21$) long, 0.31 (0.21–0.43; $n = 21$) wide, 0.013 (0.008–0.025; $n = 21$) of body length.

Molecular characterization

ITS-1 and ITS-2 were 440 and 297 bp long, respectively (GenBank accession numbers: LT717078–9). They were,

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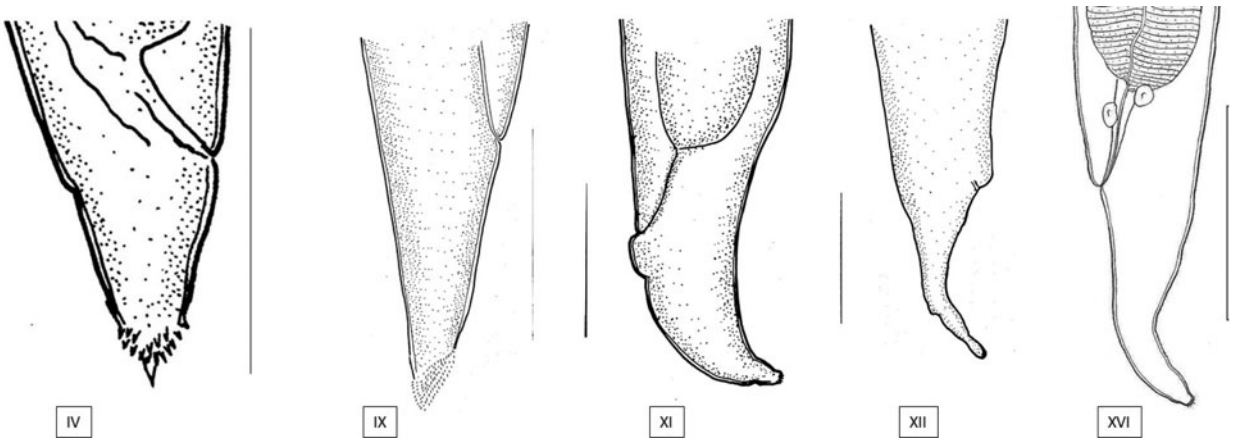

Fig. 2. Comparison of the tail morphology between *Hysterothylacium* larval types in the fourth stage of development. Roman numbers refer to the larval type. Scale bars: 0.2 mm.

respectively, 100% and 99.7% identical to previously reported *Hysterothylacium* larval type XV (GenBank accession numbers LT576348–56 (ITS-1) and LT576363–5 (ITS-2)).

Hysterothylacium reliquens (*Norris & Overstreet, 1975*)

Material examined

Twenty-eight specimens: specimen numbers 20-6, 20-15 and 20-24 (males) and 20-2, 20-3, 20-4, 20-7, 20-11, 20-12, 20-13, 20-17, 20-18 and 20-23 (females) collected from the intestine of *O. ruber*, museum accession number AHC47868; specimen numbers 1-27, 1-33, 1-34, 1-35 and 1-37 (males) and 1-3, 1-8, 1-15, 1-16, 1-17, 1-18, 1-20, 1-22, 1-30 and 1-31 (females) collected from the liver of *B. orientalis*, museum accession number AHC47869.

Description

Adults (fig. 1G–K).

Males. Cuticle annulated; alae present. Body length 23.81 (17.23–34.78; $n = 8$), width 0.60 (0.40–0.75; $n = 8$). Three labia, one dorsal, two subventrals; dorsal labium 0.17 (0.15–0.24; $n = 7$) long, 0.17 (0.12–0.25; $n = 7$) wide, subventral labium 0.16 (0.10–0.25; $n = 8$) long, 0.08 (0.05–0.13; $n = 8$) wide, interlabia 0.04 (0.03–0.05; $n = 8$) long, 0.08(0.06–0.14; $n = 8$) wide at base. Nerve ring 0.60 (0.46–0.83; $n = 8$) from anterior end. Excretory pore below the nerve ring, 0.65 (0.51–0.90; $n = 7$) from anterior end. Muscular oesophagus 2.57 (1.90–3.78; $n = 8$) long, 0.11 (0.10–0.12; $n = 8$) of body length. Ventriculus relatively round, 0.16 (0.14–0.20; $n = 8$) long. Ventricular appendix 1.16 (0.98–1.40; $n = 5$) long, 0.44 (0.36–0.52; $n = 5$) of oesophageal length. Intestinal caecum 0.30 (0.28–0.37; $n = 5$) long, 0.12 (0.08–0.15; $n = 5$) of oesophageal length and 0.28 (0.27–0.28; $n = 3$) of ventricular appendix length. Spicules blunt; spicule 1 1.56 (1.29–1.77; $n = 3$) long and spicule 2 1.76 (1.26–2.42; $n = 8$) long. Precloacal papillae 27–31 pairs, postcloacal papillae 3–4 pairs. Tail 0.19 (0.17–0.21; $n = 8$) long, 0.19 (0.15–0.22; $n = 8$) wide, 0.009 (0.006–0.011; $n = 8$) of body length.

Females. Cuticle annulated; alae present. Body length greatly variable 21.19 (5.30–48.45; $n = 20$), width 0.52 (0.15–1.08; $n = 20$). Three labia, one dorsal, two subventrals; dorsal labium 0.16 (0.05–0.33; $n = 20$) long, 0.14 (0.04–0.26; $n = 20$) wide, subventral labia 0.17 (0.05–0.33; $n = 20$) long, 0.09 (0.03–0.23; $n = 20$) wide, interlabia 0.05 (0.03–0.08; $n = 16$) long, 0.11(0.05–0.15; $n = 16$) wide at base. Nerve ring 0.53 (0.25–1.01; $n = 20$) from anterior end. Excretory pore below the nerve ring, 0.58 (0.28–1.12; $n = 19$) from anterior end. Muscular oesophagus 2.36 (0.78–4.91; $n = 20$) long, 0.12 (0.09–0.19; $n = 20$) of body length. Ventriculus relatively round, 0.16 (0.06–0.28; $n = 20$) long. Ventricular appendix 1.06 (0.42–1.94; $n = 19$) long, 0.46 (0.34–0.77; $n = 19$) of oesophageal length. Intestinal caecum 0.35 (0.18–0.56; $n = 20$) long, 0.16 (0.10–0.24; $n = 20$) of oesophageal length and 0.34 (0.20–0.51; $n = 19$) of ventricular appendix length. Tail conical, tip covered with numerous minute spinous structures, 0.33 (0.15–0.57; $n = 19$) long, 0.26 (0.09–0.48, $n = 19$) wide, 0.017 (0.012–0.028; $n = 19$) of body length.

Molecular characterization

ITS-1 and ITS-2 were 432 and 341 bp long, respectively (GenBank accession numbers: LT717080–5). They were 100% identical to ITS-1 and ITS-2 of *H. reliquens* available in GenBank (KX786289–93).

Discussion

This is the first study describing *Hysterothylacium* larval type XVI. This morphotype is in the fourth developmental stage, of which there are only four previous reports, including types IV, IX, XI and XII (Shamsi *et al*., 2013, 2015). *Hysterothylacium* type XVI in the present study can be easily distinguished from types IV, IX, XI and XII by the morphology of the tail (fig. 2). *Hysterothylacium* larval types IV and IX are distinct by having a cluster of

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Table 2. Comparison of the morphology of the *Hysterothylacium* type XV third-stage larva (L3) in the present study with previous studies. All measurements are given in millimetres.

	Li *et al.*, 2016	Shamsi *et al.*, 2016	Present study
Host	*Halieutaea stellata*	*Otolithes ruber*	*Otolithes ruber*, *Pseudorhombus arsius*, *Saurida undosquamis*, *Brachirus orientalis*
Developmental stage	L3	L3	L3
Colour	Whitish or yellowish	Whitish or yellowish	Whitish or yellowish
Body length	15.00 (8.27–19.60)	12.96 (2.26–26.83)	13.86 (5.88–20.95)
Body width	0.53 (0.34–0.68)	0.43 (0.14–0.75)	0.52 (0.25–0.68)
Labia	Weakly developed	Not developed	Not developed
Boring tooth	Very small ventral cuticular tooth	Present	Present
Nerve ring	0.36 (0.31–0.44)	0.35 (0.23–0.50)	0.26 (0.16–0.35)
Excretory pore	0.41 (0.34–0.47)	0.41 (0.22–0.58)	0.31 (0.17–0.42)
Oesophagus length	0.95 (0.58–1.13)	1.15 (0.70–1.66)	1.00 (0.58– 1.42)
Oesophagus length:total body length	–	0.07 (0.05–0.08)	0.07 (0.06–0.10)
Ventriculus	Oval to almost rounded	Almost rounded	Relatively round
Ventriculus length	0.09 (0.08–0.10)	0.12 (0.08–0.19)	0.11 (0.08–0.14)
Ventriculus width	0.10 (0.08–0.12)	0.12 (0.07–0.22)	–
Ventricular appendix	Very long	Very long	Very long
Ventricular appendix length	4.35 (2.24–6.52)	5.18 (2.87–8.83)	4.43 (1.92–6.53)
Ventricular appendix length:oesophagus length	–	4.44 (3.23–5.94)	4.35 (3.14–5.46)
Intestinal caecum	Very short, longer or as long as V	Very short	Very short
Intestinal caecum length	0.14 (0.08–0.20)	0.21 (0.09–0.30)	0.14 (0.08–0.21)
Intestinal caecum length:oesophagus length	–	0.18 (0.10–0.29)	0.15 (0.06–0.36)
Intestinal caecum length:ventricular appendix length	–	0.04 (0.02–0.07)	0.03 (0.02–0.05)
Tail	Long with a very small tip or without tip	Relatively long, conical, annulated, with a small pointy tip	Pointed with a nodular protuberance
Tail length	0.20 (0.13–0.25)	0.16 (0.10–0.28)	0.17 (0.13–0.22)
Tail length:body length	–	0.015 (0.007–0.032)	0.013 (0.008–0.025)

Table 3. Comparison of the morphometric characters of *H. reliquens* in the present study with the original report and previous reports from the same regions (measurements are given in millimetres).

Character	Norris & Overstreet, 1975	Petter & Sey, 1997	Zhao *et al.*, 2017	Present study
Hosts	*Archosargus probatocephalus, Chilomycterus schoepfi, Halichoeres bivittatus, Micropogon undulatus*	*Acanthopagrus berda, Epinephelus tauvina, Elisha elongate, Polydavtylus sextarius, Plotosus anguillaris, Pseudorhombus arsius, Synaptura orientalis, Therapon puta, Trachinotus blochi*	*Brachirus orientalis*	*Otolithes ruber Brachirus orientalis*
Locality	Mississippi, Biscayne Bay, Florida	Fish market, Kuwait city	Persian Gulf, off Basrah, southern Iraq	Khor Abdulla, Iraq
Males				
Number of specimens	23	12	11	8
Body length	21–79	40.28 (18.00–54.75)	25.1 (12.6–38.1)	23.81 (17.23–34.78)
Body width	0.52–1.88	–	0.631 (0.29–0.98).	0.60 (0.40–0.75)
Labia, length	0.15–0.38	–	0.217 (0.13–0.33)	0.17 (0.15–0.24) (dorsal)
Labia, width	0.168–0.490	–	0.21 (0.12–0.33)	0.17 (0.12–0.25)
Subventral labium length	–	–	–	0.16 (0.10–0.25)
Subventral labium width	–	–	–	0.08 (0.05–0.13)
Interlabia length	–	–	0.04 (0.02–0.07)	0.04 (0.03–0.05)
Interlabia width	–	–		0.08 (0.06–0.14)
Distance of nerve ring to anterior end	0.544–0.721	0.45–1.05	0.75 (0.49–1.08)	0.60 (0.46–0.83)
Distance of excretory pore to anterior end	0.480–1.568	0.47–1.15	0.78 (0.59–1.15)	0.65 (0.51–0.90)
Oesophageal length	2.2–9.7	4.33 (2.10–6.00)	3.37 (1.47–4.73)	2.57 (1.90–3.78)
Oesophageal length:body length	–	10.5–14.7%	13.5 (9.10–18.0)%	0.11 (0.10–0.12)
Ventriculus length	0.06–0.39 × 0.14–0.53	–	0.17 (0.07–0.30) × 0.21 (0.10–0.30)	0.16 (0.14–0.20)
Ventricular appendix length	0.940–3.360	1.71 (0.89–2.70)	1.46 (0.74–2.00)	1.16 (0.98–1.40)
Ventricular appendix: oesophageal length	–	0.24–0.59		0.44 (0.36–0.52)
Length of intestinal caecum	0.255–1.400	0.675 (0.36–1.00)	0.42 (0.20–0.65)	0.30 (0.28–0.37)
Length of intestinal caecum: length of ventricular appendix	0.17–0.42	0.26–0.62	1:2.17–4.50 (1:3.43)	0.28 (0.27–0.28)
Length of intestinal caecum: oesophageal length	–	0.12–0.20	9.98–18.8 (12.6)%	0.12 (0.08–0.15)
Spicule 1	1.24–3.38	1.86 (1.20–2.95)	0.70–2.61 (1.86)	1.56 (1.29–1.77)
Spicule 2	–	–		1.76 (1.26–2.42)
Length of spicules:body length	3–6%	3.5–7.2%	5.40–8.40 (6.60)%	4.4–5.8%
Preanal papillae	24–33 pairs	22–30	25–32 pairs	27–31 pairs
Postcloacal papillae	4–6 pairs	4–9 pairs	3–6 pairs	3–4 pairs
Tail length	0.120–0.235	2.07 (0.20–0.25)		0.19 (0.17–0.21)

Continued

Table 3. (*Cont.*)

Character	Norris & Overstreet, 1975	Petter & Sey, 1997	Zhao *et al.*, 2017	Present study
Tail width	–	–		0.19 (0.15–0.22)
Tail:total body length	–	–		0.009 (0.006–0.011)
Females				
Number of specimens	24	9	14	20
Body length	23–127	49.49 (16.3–74.00)	26.0–57.6 (40.7)	21.19 (5.30–48.45)
Body width	0.48–2.44	–	613–890 (1020)	0.52 (0.15–1.08)
Dorsal labium, length	0.164–0.510 (lips)	–	0.21–0.40 (0.28)	0.16 (0.05–0.33)
Dorsal labium, width	0.196–0.686	–	221–330 (270)	0.14 (0.04–0.26)
Subventral labia, length	–	–		0.17 (0.05–0.33)
Interlabia, length	–	–	44–110 (75.0)	0.05 (0.03–0.08; $n = 16$)
Interlabia, width	–	–		0.11 (0.05–0.15; $n = 16$)
Distance of nerve ring from anterior end	–	0.50–1.15	588–2320 (1052)	0.53 (0.25–1.01)
Distance of excretory pore to anterior end	4.80–2.019	0.55–1.32	662–2400 (1078)	0.58 (0.28–1.12; $n = 19$)
Oesophageal length	2.4–11.6	5.71 (1.70–7.96)	2.45–6.71 (3.98)	2.36 (0.78–4.91)
Oesophageal length:body length	–	9.4–13.1%	6.00–12.1 (9.85)%	0.12 (0.09–0.19)
Ventriculus length	0.076–0.421 × 0.136–0.588	–	147–380 (206) long, 172–450 (267) wide	0.16 (0.06–0.28)
Ventricular appendix length	0.880–4.450	1.84 (0.55–2.70)	1.00–2.20 (1.62)	1.06 (0.42–1.94; $n = 19$)
Length of ventricular appendix: oesophageal length	–	0.26–0.50		0.46 (0.34–0.77; $n = 19$)
Length of intestinal caecum	0.26–0.18	0.93 (0.47–1.40)	368–650 (469)	0.35 (0.18–0.56)
Length of intestinal caecum: length of ventricular appendix	0.20–0.56	0.42–0.91	1:1.71–7.14 (1:3.32)	0.34 (0.20–0.51)
Length of intestinal caecum: oesophageal length	–	0.26–0.29	7.0–24.0 (12.7)%	0.16 (0.10–0.24)
Distance of vulva from anterior end	7.6–33.0	–	5.32–17.8 (10.5)	14.3 (11.8–16.8)
Distance of vulva from anterior end:body length	29–34%	33–42% ($n = 3$)	20.5–45.3 (28.4)%	34 (31–39)%
Tail length	0.300–0.686	0.45 (0.19–0.55)	363–500 (423)	0.33 (0.15–0.57; $n = 19$)
Tail width	–	–		0.26 (0.09–0.48, $n = 19$)
Tail:body length	–	–		0.017 (0.012–0.028; $n = 19$)

spines, and types XI and XII can be differentiated morphologically based on the width of the tail region, being very narrow in type XII and being fleshier in type XI. In addition to the morphological differences, ITS sequence data support the distinction of *Hysterothylacium* larval type XVI from types IV, IX, XI and XII. Interestingly, alignment of the ITS-1 and ITS-2 sequence data of *Hysterothylacium* larval type XVI in the present study with those deposited in GenBank shows that they are almost identical (99.5–100% similarity) with adult *H. persicum* (GenBank accession numbers LT576366, LT576367, LT576368 (ITS-1) and LT576369, LT576370, LT5 76371 (ITS-2)) and *Hysterothylacium* larval type XIV (GenBank accession numbers LN651105–LN651107), which is a third-stage larva. Therefore, the present study contributed to elucidating the partial life cycle of *H. persicum* among various hosts and its distribution, which seems to be wide, at least from New Caledonia in the South Pacific to the most western corner of the Persian Gulf, off Iraqi coasts in the northern hemisphere.

Another *Hysterothylacium* larval type in the present study was *Hysterothylacium* type XV, which had been described previously for the first time from the East and South China Sea (Li *et al.*, 2016) and later from the area joining the Persian Gulf and the Gulf of Oman (Shamsi *et al.*, 2016). In the present study, we report three new hosts, *P. arsius*, *S. undosquamis* and *B. orientalis*, for this larval type. Comparison of morphological characters between specimens examined in the present study and those in previous studies (table 2) showed that they belong to the same larval type. This is also supported by ITS-1 and ITS-2 sequence data, which were, respectively, 100% and 99.7% identical to previously reported *Hysterothylacium* larval type XV (Shamsi *et al.*, 2016), GenBank accession numbers LT576354 (ITS-1) and LT576363 (ITS-2).

Hysterothylacium reliquens was first described by Norris & Overstreet (1975) from *Archosargus probatocephalus* (type host) and other fishes of the northern Gulf of Mexico and southern Florida. The species was later reported sporadically from other parts of the world, including from the Persian Gulf off Kuwait and Iraq (Petter & Sey, 1997; Zhao *et al.*, 2017). Comparison of morphological characteristics of taxonomic significance among *H. reliquens* specimens in the present study and those in the previous studies (table 3) does not show a significant difference and, as suggested previously (Zhao *et al.*, 2017), it seems that this species has a wide geographical distribution across continents. In the present study we report *O. ruber* as a new host for *H. reliquens*.

In conclusion, the present study showed the presence of at least three members of the genus *Hysterothylacium* in Iraqi fish. The detailed morphological characterization, along with characterization of the ITS-1 and ITS-2 regions of these taxa, can be useful for any future taxonomical and systematics studies of these parasites in the region and beyond.

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Conflict of interest

None.

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