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Morphological and molecular studies of *Hysterothylacium thalassini* third-stage larvae (Ascaridida, Raphidascarididae) in the greater lizardfish *Saurida tumbil*

[*Estudos morfológicos e moleculares das larvas de terceiro estágio de Hysterothylacium thalassini (Ascaridida, Raphidascarididae) no peixe-lagarto Saurida tumbil*]

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ABSTRACT

Hysterothylacium species are perhaps the most abundant and diverse group of marine ascaridoids. Identification of larval stages at specific levels is very problematic. This study describes the occurrence of *Hysterothylacium* larvae parasitizing the peritoneal cavity and mesenteries of *Saurida tumbil* purchased from fish markets in Saudi Arabia. Fish were visually inspected for nematodes using micro- and macroscopic examinations. Nematodes were assigned to genus level based on morphology and identified at specific level by sequence analyses of *18S rRNA* and *COI* genes. Morphological examination by light microscopy showed that worms identified as third-stage larvae (L3) belonged to genus *Hysterothylacium* Ward and Magath, 1917. COI sequences from host confirmed identity of host as *S. tumbil* as it resulted in identical sequences from *S. tumbil* in GenBank. Identity of L3 based on 18S rDNA sequences confirmed their identity as *H. thalassini* and showed high similarity to sequences in GenBank. A unique hitherto sequences of L3 related to COI region have been reported herein. This is the first record of *Hysterothylacium* L3-stage parasitizing lizardfish in Saudi Arabia. Therefore, this study represents the importance of a combination of morphological and molecular tools for taxonomy and systematics of ascaridoids at specific level and confirming its host identity.

Keywords: Ascaridoidea, larvae, identification, *Saurida* species, Saudi Arabia

RESUMO

As espécies de Hysterothylacium são talvez o grupo mais abundante e diversificado de ascaridóides marinhos. A identificação dos estágios larvais em nível específico é muito problemática. Este estudo descreve a ocorrência de larvas de Hysterothylacium parasitando a cavidade peritoneal e os mesentérios de Saurida tumbil, comprados em mercados de peixe na Arábia Saudita. Os peixes foram inspecionados visualmente em busca de nematoides por meio de exames micro e macroscópicos. Os nematoides foram classificados em nível de gênero com base na morfologia e identificados em nível específico por meio de análises de sequência dos genes 18S rRNA e COI. O exame morfológico por microscopia de luz mostrou que os vermes identificados como larvas de terceiro estágio (L3) pertenciam ao gênero Hysterothylacium Ward e Magath, 1917. As sequências de COI do hospedeiro confirmaram a identidade do hospedeiro como S. tumbil, pois resultaram em sequências idênticas de S. tumbil no GenBank. A identidade da L3 com base nas sequências de rDNA 18S confirmou sua identidade como H. thalassini e mostrou alta similaridade com as sequências do GenBank. Uma sequência única de L3 relacionada à região COI foi relatada aqui. Esse é o primeiro registro do estágio L3 do Hysterothylacium parasitando peixes-lagarto na Arábia Saudita. Portanto, este estudo representa a importância de uma combinação de ferramentas morfológicas e moleculares para a taxonomia e a sistemática de ascaridóides em nível específico e para a confirmação da identidade de seu hospedeiro.

Palavras-chave: Ascaridoidea, larvas, identificação, espécies de Saurida, Arábia Saudita

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INTRODUCTION

The family Synodontidae (Aulopiformes), commonly known as lizardfish, inhabits both tropical and subtropical marine waters (Kalhoro *et al*., 2015). Lizardfish has a high protein content with a significant nutritional value (Meena *et al*., 2015). Of the various species of lizardfish, the greater lizardfish *Saurida tumbil* (Bloch, 1795), is distributed in the Red Sea, the Eastern coast of Africa, the Persian Gulf, the Arabian Sea, East to Southeast Asia and Australia (Froese and Pauly, 2018). Fish are vulnerable to various parasitic infections that cause potent problems in human health (Dela Cruz *et al*., 2022).

Nematodes of the family Raphidascarididae (Ascaridida), and a species of the genera *Hysterothylacium* Ward and Magth, 1917 and *Raphidascaris* (*Ichthyascaris*) Wu, 1949, are the most cosmopolitan marine ascaridoid in various fish species and the heavy infection of these parasites leads to reduce the aesthetical appeal of fish products (Bao *et al*., 2021). The genus *Hysterothylacium* includes 91 accepted species, three taxon inquirendum, two nomen dubia, and ten unaccepted species (WoRMS, 2023) and is considered one of the largest of the ascaridoid genera parasitizing fish. The adult stages of *Hysterothylacium* are found parasitizing the alimentary canal of marine, estuarine, and freshwater fish (final host) (Li *et al*., 2007), while larvae can parasitize various tissues of invertebrates (1st intermediate host) and fish (2nd intermediate or paratenic host) (Køie, 1993). Human infections with *Hysterothylacium* species are not common but are recorded previously by Yagi *et al*. (1996) and González-Amores *et al*. (2015).

Morphological features of ascaridoid nematodes are insufficient for the species identification. Recently, molecular studies have been proven to be useful for the accurate identification of *Hysterothylacium* species using DNA sequencing of small subunit (18S) ribosomal RNA (Knoff *et al*., 2012; Abdel-Ghaffar *et al*., 2015; AlGabbani *et al*., 2021), internal transcribed spacer (ITS) region (Shamsi *et al*., 2010; Amor *et al*., 2011; Borges *et al*., 2012; Knoff *et al*., 2012; Liu *et al*., 2013; Pekmezci *et al*., 2014; Shamsi *et al*., 2015; Pantoja *et al*., 2016; Li *et al*., 2017; Ghadam *et al*., 2018; Simsek *et al*., 2018; Cavallero *et al*., 2019; AlGabbani *et al*., 2021; Bannai *et al*., 2021; Bannai and Jori, 2022; De Benedetto *et al*., 2022; Utami *et al*., 2022) and the mitochondrial cytochrome c oxidase subunit 1 (*COI*) (AlGabbani *et al*., 2021) and 2 (COII) genes (Borges *et al*., 2012; Knoff *et al*., 2012; Pekmezci *et al*., 2014; Pantoja *et al*., 2016; De Benedetto *et al*., 2022).

Although numerous studies on marine fish parasites have been conducted, little is known about ascaridoid nematodes in lizardfish (Xu *et al*., 2014; AlGabbani *et al*., 2021). Therefore, the present study aimed to determine the prevalence of ascaridoid nematodes infecting *Saurida tumbil* from the Red Sea coast (Saudi Arabia) and characterize these parasites using morphological and genetic analyses focusing on the proper identification at the specific level. Moreover, identify the host fish that corresponds with ascaridoid nematodes molecularly.

MATERIALS AND METHODS

Fish sampling. The greater lizardfish *Saurida tumbil* (n=60) was purchased, from February to June 2023, from the fish markets of Jeddah city along the Red Sea (Saudi Arabia). Fish were transported to the Laboratory of Parasitology Research (College of Science, King Saud University) for further examination.

Parasitological study. After dissection, the visceral cavity and digestive tract of each fish were inspected macroscopically for the presence of endoparasites. Worms were picked up and washed several times in physiological saline (0.9%). Anterior and posterior parts of the isolated worms were fixed in 70% ethanol for morphological studies, then cleared in lactophenol, mounted on slides with glycerol gelatin, and photographed using a Leica DM 2500 microscope (NIS ELEMENTS software, version 3.8). The middle parts of these worms were preserved in 96% ethanol for molecular analysis. Measurements (the mean, followed by the range in parentheses) were given in millimeters (mm) using ImageJ 1.53e software (Wayne Rasband and contributors, National Institute of Health, USA). Parasite identification was based on the diagnostic keys of Berland (1961). Epidemiological parameters including prevalence and mean intensity of infection were calculated according to the equations of Bush *et*

al. (1997). Moreover, a small portion of the hepatopancreas of the host fish was removed, washed in saline, and then preserved in 96% ethanol for molecular identification.

Molecular study. Genomic DNA was extracted from ethanol-preserved parasite and host samples (hepatopancreas) using QIAamp® DNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. PCR targeting partial nuclear small subunit ribosomal RNA (*18S rRNA*) gene for parasite identification and mitochondrial cytochrome c oxidase I subunit (*COI*) for parasite and host identification. The *18S rRNA* was amplified using the forward primer (Nem 18SF, 5′-CGC GAA TRG CTC ATT ACA ACA GC-3′) and the reverse primer (Nem 18SR, 5′-GGG CGG TAT CTG ATC GCC-3′) (Floyd *et al*., 2005). The *COI* gene (for the parasite) was amplified using the forward primer (LCO1490, 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and the reverse primer (HC02198, 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) published by Folmer *et al*. (1994). For the host, *COI* was amplified using a forward primer (Fish1F: 5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′) and a reverse primer (Fish1R: 5′-TAG AC TTC TGG GTG GCC AAA GAA TCA-3′) as published by Ward *et al*. (2005). Amplification fragments were analyzed by electrophoresis in 1.5% w/v agarose gel (Sigma-Aldrich, Missouri, USA) and their sizes were determined by comparison with a 100 bp DNA ladder marker (Fermentas, Lithuania). PCR products were sequenced in Macrogen (Seoul, South Korea) using the same primers as for PCR. The *18S rRNA* and *COI* sequences were deposited in GenBank™ and then compared with those available in the NCBI database. For the phylogenetic study, the sequence data were installed into MEGA X (Kumar *et al*., 2018). Phylogenetic trees were inferred using maximum parsimony with appropriate models. Bootstrap resampling was computed for 1000 replicates.

RESULTS

Third stage *Hysterothylacium* larvae were isolated from fifty-three lizardfish among the total of sixty specimens with a mean prevalence of 88.33%. Intensity of larvae per fish ranged from 10 to 15. These nematodes were found usually as encapsulated larvae in the peritoneal cavity and mesenteries (Figure 1 A, B). The examined fish showed no external visible signs of disease.

Morphological description (Figure 1 C-K and Table 1). The body of the recovered larvae was cylindrical, attenuated at both ends, and measured 10.03 (7.11-18.45) long and 0.62 (0.47-0.75) wide. The cuticle had transverse striations that extended from the cephalic region before the anus. The anterior extremity of poorly developed labia provided with prominent boring teeth. Four labial papillae (two dorsolateral and two ventrolateral) surrounded the triradiate mouth opening. The nerve ring and excretory pore are located at 0.29 (0.17-0.41) and 0.34 (0.19-0.49) from the anterior end, respectively. The oesophagus had an anterior muscular part (1.25 (0.70-1.63) long) and a glandular ventriculus with an oblique oesophago-intestinal junction. The intestinal caecum is shorter than the ventricular appendix and measured 0.16 (0.11-0.23) long. Rectum is surrounded by four rectal glands and opens by an anal opening. The tail was conical with a tip bent dorsally and decorated with a nodular protuberance and measured 0.14 (0.11-0.20) long. The larvae recovered in the present study were identified as *Hysterothylacium thalassini*.

Molecular analysis (Figures 2 and 3). DNA from the lizard fish resulted in amplification of ~750 bp of the *COI* region using primers Fish F and Fish R. DNA sequences from 5 individuals were generated and they were deposited in GenBank and given the accession numbers OR681256-OR681260. Sequences are identical with only one mutation at position 402 of the alignment where A in the first three sequences is replaced by a G on sequences OR681259 and OR681260.

This mutation is shared with the other 4 sequences from India included in the present study. Additionally, the sequence KR105892 from Kerala, India showed mutation at position 288 and it was a G whereas it was an A in all other sequences included in analysis including those of the present study. Sequences from the present study were 99.83% identical to those sequences of *Saurida tumbil* from India (Kerala (KR105898, KR105892 and KR105895 and Lucknow EF609600). Pylogenetic trees resulting from analysis grouped the sequences obtained in the present study with those of *S. tumbil* from

India with 100 and 99 bootstrap values on both Maximum Likelihood (ML) and Neighbor Joining (NJ) analyses respectively (Figure 2). Hence confirming the morphological description of the host species. Other species of the genus *Saurida* are distinct from *S. tumbil*.

The PCR resulting from the amplification of the *18S rRNA* of the parasite resulted in ~890 bp and they were aligned with other 18S rRNA sequences from related species. Six sequences were generated and deposited in GenBank and were given the accession numbers OR681261-OR681266. Sequences were found to be identical with only one sequence (OR681261) showing a mutation at position 803 of the alignment (at transition G whereas it is found as A in all other sequences).

Figure 1. (A and B) Third-stage larvae (white arrow) for the nematode parasite, *Hysterothylacium thalassini*, in the peritoneal cavity of *Saurida tumbil*. (C) Whole mount preparation. (D-K) High magnifications for different body parts including (D, E) Anterior part. (F) Excretory pore. (G) Transverse annulations of cuticle. (H) Intestinal caecum and ventriculus with its appendix. (I-K) Posterior part. Note: BT, boring tooth; MO, mouth opening; CP, cephalic papillae; OE, oesophagus; EP, excretory pore, EC, excretory canal; C, cuticle; IC, intestinal caecum; V, ventriculus; VA, ventricular appendix; IN, intestine; RG, rectal gland; R, rectum; AO, anal opening; T, tail; Black arrow, nodular protuberance at the tail tip.

Two other sequences (MF072702 and OQ627197) were also found to be identical to the sequences OR681262-OR681266. The identity between the two sequences and five sequences reported in the present study (OR6812620-OR681266) was 100% in 839 bases. Sequences obtained in the present study clustered with two sequences, one from *H. thalassini* (MF072702) and the other from an unidentified nematode (MF072702) Ascaridoidea gen. n. sp. with high bootstrap values on both ML and NJ as seen in Figure (3). Other species of *Hysterothylacium* are grouped separately.

Sequences from the *COI* region of third larval stage resulted in 3 unidentical sequences which are deposited in GenBank with the accession numbers OR689276-OR689278. There were no sequences related to *H. thalassini* or any other *Hysterothylacium* species available in GenBank to compare our sequences with. There were seven mutations seen in the sequences and all of them are transitions. The Sequence OR689277 showed one mutation from sequence OR698276 at position 239 of the alignment. Whereas sequence OR689278 showed 6 mutations at positions 57, 225, 417, 483, 525, and 555. The sequences of the amino acids were found to be identical except for the sequences OR689277 where the amino acid at position 80 was alanine whereas on the other two sequences, it was valine.

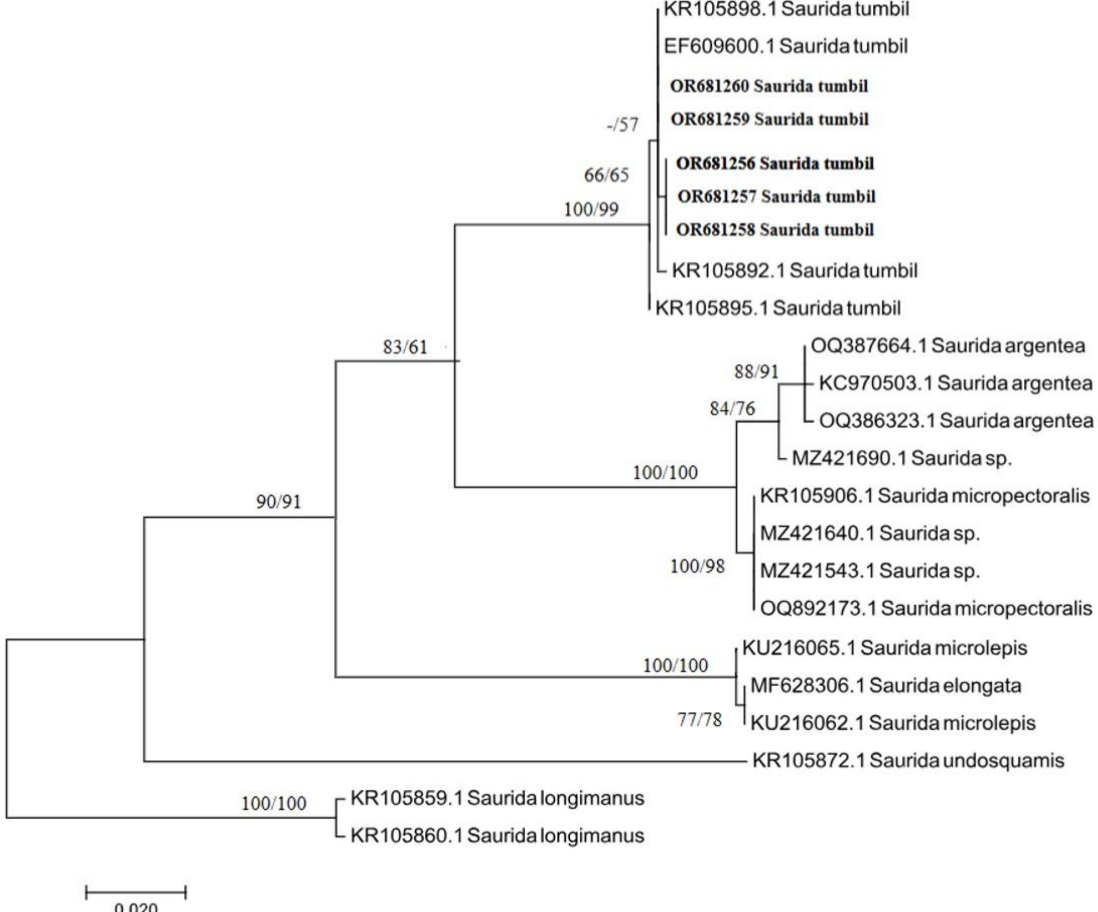

Figure 2. A consensus phylogenetic tree constructed with maximum likelihood (ML) and Neighbor Joining (NJ) methods, showing phylogenetic relationships between *Saurida tumbil* (5 sequences) and 18 related taxa in NCBI GenBank. The ML and NJ trees are inferred from the partial COI sequence data generated from the *S. tumbil* detected in the present study (OR681256-OR681260) shown in bold and related taxa. Numbers indicated at branch nodes are bootstrap values (ML/NJ). Only bootstraps > 50% are shown.

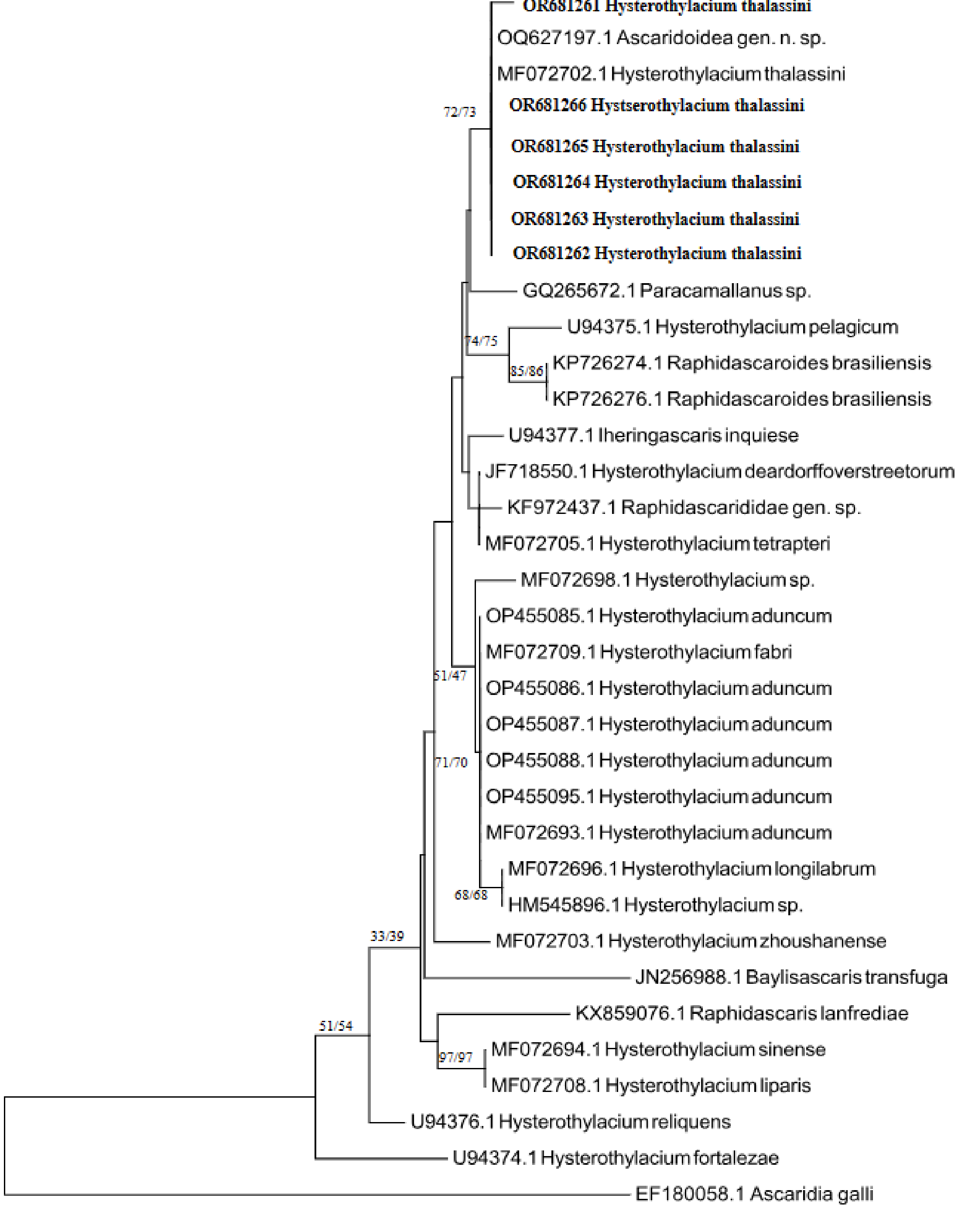

Figure 3. A consensus phylogenetic tree constructed with maximum likelihood (ML) and Neighbor Joining (NJ) methods, showing phylogenetic relationships between *Hysterothylacium thalassini* (6 sequences) and 27 related taxa in NCBI GenBank with *Ascaridia galli* as an outgroup. The ML and NJ trees are inferred from the partial 18S rDNA sequence data generated from the *H. thalassini* detected from *Saurida tumbil* (OR681261-OR286266) shown in bold with related taxa. Numbers indicated at branch nodes are bootstrap values (ML/NJ).

Table 1. Comparison of morphometric characters of the recovered third-stage larvae of *Hysterothylacium* species with those described previously from various fish species

Reference	Host fish	Body		Location from anterior extremity		Ventricular appendix	Intestinal caecum	Tail length
		Length	Width	Nerve ring	Excretory pore			
Deardorff and Overstreet 1981	*Scomberomorus maculatus*	1.7-3.5	0.04-0.08	-	-	0.10-0.27	0.06-0.08	0.07-0.15
	Micropogonias undulatus	4.0-9.0	0.067-0.25	-	-	0.27-0.59	0.12-0.55	0.08-0.15
Moravec *et al.*, 1997	*Percichthys trucha*	7.02-7.34	0.136-0.19	0.25-0.28	0.29-0.30	0.53-0.60	0.45-0.51	0.08-0.12
Novone *et al.*, 1998	*Themisto gaudichaudii*	4.95-12.37	0.12-0.30	0.18-0.28	0.20-0.39	0.30-0.57	0.39-0.68	0.08-0.18
	Engraulis anchoita	4.91-15.84	0.09-0.33	0.21-0.36	0.25-0.46	0.31-0.61	0.38-0.91	0.07-0.21
	Merluccius hubbsi	9.49-18.86	0.25-0.34	0.25-0.40	0.28-0.45	0.48-0.71	0.66-1.50	0.10-0.19
Felizardo *et al.*, 2009	*Paralichthys isosceles*	10.1 (3.62-16.7)	0.25 (0.11-0.40)	0.12-0.46 (0.29)	0.36 (0.25-0.46)	0.86 (0.35-1.37)	0.18 (0.05-0.32)	0.20 (0.10-0.32)
Li *et al.*, 2012	*Siganus fuscescens*	10.2 (8.20-13.1)	0.30 (0.19-0.39)	0.39 (0.29-0.49)	0.42 (0.31-0.53)	0.70 (0.48-0.84)	0.13 (0.10-0.19)	0.19 (0.14-0.24)
Al-Zubaidy *et al.*, 2012	*Epinephelus guttatus, E. tauvina, Sphyraena barracuda, S. jello, Lutjanus gibbus, Pristipomoides filamentosus, Abalistes sterllaris, Carangoides bajad*	9.2 (4.5-10.9)	0.14 (0.09-0.20)	-	-	0.52 (0.33-0.75)	0.17 (0.06-0.20)	0.13 (0.10-0.22)
Shamsi *et al.*, 2013	*Lutjanus argentimaculatus, L. caronotatus, L. fulviflammus*	13.3 (11.4-15.1)	0.29 (0.09-0.32)	0.33 (0.31-0.36)	0.41 (0.33-0.48)	1.3 (1.1-1.4)	0.25 (0.20-0.30)	0.11 (0.09-0.12)
	Engraulis australis, Scomber neopilchardus, Seriola hippos, Seriola lalandi, Sillago flindersi, Scomber australasicus	6.5 (3.5-9.2)	0.20 (0.08-0.30)	0.26 (0.16-0.34)	0.28 (0.08-0.36)	0.42 (0.22-0.54)	0.40 (0.16-0.60)	0.14 (0.08-0.22)
	Sphyraena novaehollandiae	5.8 (3.9-7.1)	0.23 (0.16-0.28)	0.27 (0.16-0.40)	0.33 (0.19-0.48)	0.81 (0.44-1.36)	0.22 (0.10-0.26)	0.18 (0.13-0.26)
Ribeiro *et al.*, 2014	*Chaetodipterus faber*	13.10 (12.60-13.70)	0.34 (0.34-0.35)	0.30 (0.30-0.31)	-	1.01 (0.80-1.22)	0.47 (0.35-0.60)	0.17 (0.14-0.21)
	Trachinotus carolinus	14.86 (11.16-19.02)	0.30 (0.21-0.43)	0.30 (0.16-0.43)	-	0.75 (0.48-1.08)	0.31 (0.11-0.45)	0.20 (0.12-0.27)
Andrade-Porto *et al.*, 2015	*Arapaima gigas*	2.53 (2.14-2.87)	0.10 (0.09-0.12)	0.11 (0.10-0.13)	0.12 (0.11-0.13)	0.85 (0.62-0.97)	0.09 (0.05-0.16)	-
Fontenelle *et al.* 2015	*Selene setapinnis*	6.05 (4.50-7.0)	0.19 (0.14-0.22)	0.20 (0.16-0.24)	-	0.53 (0.45-0.60)	0.18 (0.15-0.21)	0.17 (0.12-0.20)
Shamsi *et al.*, 2015	*Gymnocranius euanus, G. superciliosus, Rastrelliger kanagurta, Sphyraena forsteri*	4.78 (3.13-6.50)	0.24 (0.15-0.33)	0.22 (0.18-0.28)	0.32 (0.20-0.44)	0.48 (0.40-0.73)	0.14 (0.08-0.20)	0.14 (0.10-0.18)
	Abalistes stellatus	9.88	0.31	0.30	0.62	0.70	0.24	-
	Herklosichthys quadrimaculatus	4.93 (3.88-5.75)	0.18 (0.15-0.25)	0.27 (0.22-0.33)	0.37 (0.31-0.43)	0.39 (0.24-0.54)	0.18 (0.13-0.26)	-

Reference	Host fish	Body		Location from anterior extremity		Ventricular appendix	Intestinal caecum	Tail length
		Length	Width	Nerve ring	Excretory pore			
Li *et al*., 2016	*Halieutaea stellate*	15.00 (8.27-19.60)	0.53 (0.34-0.68)	0.36 (0.31-0.44)	0.41 (0.34-0.47)	4.35 (2.24-6.52)	0.14 (0.08-0.20)	0.20 (0.13-0.25)
Shamsi *et al*. 2016	*Otolithes ruber*	12.96 (2.26-26.83)	0.43 (0.14-0.75)	0.35 (0.23-0.50)	0.41 (0.22-0.58)	5.18 (2.87-8.83)	0.21 (0.09-0.30)	0.16 (0.10-0.28)
Pantoja *et al*., 2016	*Priacanthus arenatus*	6.4 (3.7-8.5)	0.16 (0.10-0.21)	0.29 (0.21-0.40)	0.36 (0.29-0.48)	0.53 (0.38-0.66)	0.17 (0.12-0.24)	0.24 (0.13-0.55)
	Elops saurus	-	0.15	0.18	0.21	0.53	0.05	0.13
Ghadam *et al*., 2018	*Epinephelus areolatus, Otolithes ruber, Pseudorhombus arsius, Saurida undosquamis, Brachirus orientalis*	13.86 (5.88-20.95)	0.52 (0.25-0.68)	0.26 (0.16-0.35)	0.31 (0.17-0.42)	4.43 (1.92-6.53)	0.14 (0.08-0.21)	0.17 (0.13-0.22)
Khalifa *et al*., 2019	*Lethrinus mehsena, Parupeneus forsskali, L. variegates*	3.93 (2.71-6.20)	0.12 (0.08-0.19)	0.135 (0.09-0.20)	-	0.34 (0.24-0.50)	0.07 (0.04-0.11)	0.13 (0.11-0.17)
	Parupeneus forsskali	4.60	0.16	0.273	0.34	0.59	0.14	0.15
	Rhabdosargus haffara, Parupeneus forsskali	6.54-8.86	0.23-0.27	0.24-0.28	0.34-0.58	0.46-0.63	0.16-0.22	0.14-0.18
	Parupeneus cyclostomus	4.49	0.16	0.22	0.30	0.24	0.13	0.12
AlGabbani *et al*., 2021	*Argyrops spinifer*	6.20 (5.32-8.97)	0.48 (0.15-0.64)	0.21 (0.09-0.22)	0.31 (0.11-0.39)	2.28 (2.31-3.34)	0.19 (0.18-0.20)	0.18 (0.16-0.19)
Gelen and Pekmezci 2023	*Mullus barbatus*	10.0 (10.0-11.0)	0.17 (0.16-0.19)	0.27 (0.26-0.28)	0.37 (0.36-0.39)	0.90 (0.84-0.98)	0.07 (0.06-0.07)	0.12 (0.10-0.16)
	Engraulis encrsicolu, Trachurus trachurus, Mullus barbatus, Merlangius merlangus	11.0 (7.0-12.0)	0.21 (0.20-0.22)	0.30 (0.28-0.34)	0.41 (0.37-0.44)	0.51 (0.41-0.71)	0.55 (0.45-0.80)	0.14 (0.11-0.20)
Serrano *et al*., 2023	*Pomatomus saltatrix*	8.0 (1.0-9.63)	0.18 (0.15-0.32)	0.48 (0.16-0.65)	0.55 (0.20-0.69)	0.58 (0.25-0.69)	0.07 (0.03-0.10)	0.26 (0.16-0.32)
	Pagrus pagrus	9.67 (1.78-11.64)	0.26 (0.12-0.40)	0.52 (0.34-0.66)	0.58 (0.22-0.75)	0.78 (0.29-1.02)	0.09 (0.05-0.20)	0.33 (0.21-0.45)
Present study	*Saurida tumbil*	10.03 (7.11-18.45)	0.62 (0.47-0.75)	0.29 (0.17-0.41)	0.34 (0.19-0.49)	4.18 (2.04-5.67)	0.16 (0.11-0.23)	0.14 (0.11-0.20)

DISCUSSION

The Raphidascaridae family shows a worldwide distribution and parasitizes various fish species. Species of *Hysterothylacium* are the most common and diverse group of marine ascaridoids (Guo *et al*., 2014). Marine fish could act as both the paratenic and/or intermediate and definitive hosts of *Hysterothylacium* species. Prevalence of infection in their fish hosts varies greatly, depending on the hosts and age, as well as on the fishing area (Al-Salim and Ali, 2010; Shamsi *et al*., 2016; Zhao *et al*., 2017). In this study, fifty-three lizardfish (88.33%) were found to be naturally infected with larval raphidascarids within the genus *Hysterothylacium*. The present high prevalence is consistent with the previous data *Hysterothylacium* species reported by Smith (1983, 81.2%) in euphausiids in the North-East Atlantic and the northern North Sea, Bicudo *et al*. (2005, 97.5%) in *Prionotus punctatus* from the municipality of Angra dos Reis, State of Rio

de Janeiro, Li *et al.* (2007, 78.3%) in *Pseudorhombus cinnamoneus* from Yellow Sea (Shandong Province, China), Felizardo *et al.* (2009, 100%) from *Paralichthys isosceles*, Guo *et al.* (2014, 100%) in the Tanaka's snailfish *Liparis tanakae*, Andrade-Porto *et al.* (2015, 98%) in *Arapaoma gigas* from South America, Bannai *et al.* (2021, 91.11%) from Iraqi marine water fish, and Serrano *et al.* (2023, 75%) in *Pomatomus saltatrix* and *Pagrus pagrus* from State of São Paulo, Brazil. However, this ratio is higher than those mentioned for *Hysterothylacium* larvae by Sánchez-Ramiréz and Vidal-Martinéz (2002, 13-33%) in *Trachinotus carolinus*, Li *et al.* (2012, 12.5%) in *Siganus fuscescens*, and Kuraiem *et al.* (2017, 66.7%) in *Priacanthus arenatus*.

In this study, larvae were encysted within the peritoneal cavity and mesenteries of a fish host, which agreed with the previous data of Andersen (1993), Køie (1993) and Shih and Jeng (2002) reported that species of *Hysterothylacium* parasitic as adults in the gut of fish, whereas larvae have been regularly encapsulated throughout the viscera and penetrated the tissues of fish and various invertebrates. These larvae could easily reach humans through the ingestion of raw or improperly cooked fish meat and cause zoonotic character (anisakidosis) (Yagi *et al.*, 1996; Felizardo *et al.*, 2009; Cavalcanti *et al.*, 2012; Fontenelle *et al.*, 2013; Andrade-Porto *et al.*, 2015). Clinical signs depend on the site where the larvae are deposited, but it generally causes abdominal pain and vomiting, as well as some allergic reactions (Fumarola *et al.*, 2009).

Combination of morphological features including the body size, the shape of the anterior extremity with a boring tooth, the location of the excretory pore at or near the level of the nerve ring, the ratio of the intestinal caecum relative to the ventricular appendix, and the morphology of tail extremity, identify the nematodes collected from lizardfish as third-stage larvae (L3) belonging to the genus *Hysterothylacium.* This finding is consistent with Deardoff and Overstreet (1981) who reported that worms with boring teeth are considered L3 larvae of *Hysterothylacium* species. Previous studies have been conducted on the morphotypes of *Hysterothylacium* larva types (I and II) in various fish species. Our larval stages are morphologically distinct based on the presence of boring tooth, the smaller length ratio of the intestinal caecum in comparison to the ventriculus appendix, and the presence of nodular protuberance at the tail tip, which is consistent with the data obtained by Moravec *et al.* (1997), Felizardo *et al.* (2009), Al-Zubaidy *et al.* (2012), Shamsi *et al.* (2013) for III and VIII larvae, Ribeiro *et al.* (2014), Andrade-Porto *et al.* (2015), Ghadam *et al.* (2018), Shamsi *et al.* (2015) for VI and XIII larvae, Khalifa *et al.* (2019) for VI larvae, AlGabbani *et al.* (2021), and Gelen and Pekmezci (2023) for III and VIII larvae to be recognized as L3-stage *Hysterothylacium* larval type I.

The L3 larvae, in this study, are similar to the description of Ghadam *et al.* (2018), indicating that the larvae agree with the description presented by other authors, who have identified it such as *Hysterothylacium* larval type XV from *Saurida undosquamis* in Iraqi waters. Morphometric variations with extreme range were observed with other comparable *Hysterothylacium* larvae, this agreed with Pantoja *et al.* (2016) hypothesized that the issue depends upon host attributes (including body and gut size), the intensity of the parasite burdens and the frame of development, where L3 may be closer to the second or latter to the fourth stage larvae. Based on previous studies, it appears that L3-stage *Hysterothylacium* larvae show a broad host-specificity. The present study provides the first report in Saudi Arabia of *Saurida tumbil* harbored larval raphidascarids. According to Li *et al.* (2012), Ghadam *et al.* (2018), Shamsi *et al.* (2018), Hossen and Shamsi *et al.* (2019), and Gelen and Pekmezci (2023) it is impractical and problematic to identify larval morphotypes at the specific level using morphological characters alone, thus molecular tools were used for the exact identification of species.

DNA sequences from the *COI* region of the host (*Saurida tumbil*) grouped with sequences from the same host from different regions in India (Sequences included KR105892, KR105895, KR105898, EF609600). Indicating that the distribution of the *S. tumbil* is reaching the Red Sea and confirming the identity of the host in which the third larval stage of the parasite was found. Phylogenetic analyses of partial 18S rRNA sequences obtained from the third larval stage (L3) in the present study revealed that the sequences grouped with those from the nematode parasite *H. thalassini*. Since only one sequence

of the 18S rRNA belonging to *H. thalanssini* is available in GenBank the identity of sequences was 99.9-100%. The other sequences that were obtained from an unidentified worm (MF072702) *Ascaridoidea* gen. n. sp. also showed the same similarity to sequences obtained in the present study. It is unclear whether the worm they are claiming as a new genus has ever been published or described. We strongly suggest that they are probably dealing with the same parasites till full morphological description is revealed. Interestingly, there were no sequences related to the *COI* gene from the *H. thalassini*, hence our sequences in the present study are the first published sequences from *H. thalassini*. Interestingly, there are only two sequences from different species of the genus *Hysterothylacium*. Those are *H. aduncum* (ON514619) from *Engraulis encrasicolus* in Spain (Rodríguez-Romeu *et al*., 2022) and *H. reliquens* (MZ148789) from *Argyrops spinifer* in Saudi Arabia. The sequence divergence between these sequences and the sequences from the present study was divergent as they showed.

CONCLUSION

It is concluded from the present study that the parasite ascaridoid species found in *S. tumbil* was *Hysterothylacium* type I larvae and identified as *Hysterothylacium thalassini* third-stage larvae with unique 18S rDNA sequences and a hitherto sequence to *COI* gene and having new locality and host records in the Red Sea (Saudi Arabia). Future studies are required to explore the remaining parasitic taxa infecting different tissues of lizardfish.

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