

MOLECULAR CHARACTERIZATION OF ANISAKID NEMATODES
HYSTEROETHYLACIUM SPECIES FROM JAPANESE THREADFIN
BREAM *NEMIPTERUS JAPONICUS* (BLOCH, 1791) (PERCIFORMES,
NEMIPERIDAE) FROM IRAQI MARINE WATER FISH

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ABSTRACT

The present study provides a new insight into valuable information on the diverse structure of the Anisakid population and discusses the limited species richness in the *Nemipterus japonicus* (Bloch, 1791) (Perciformes, Nemiperidae). The fishing area consists of various locations in the Arabian Gulf (29°58' 03" 00" N 48°28' 02" 00" E). A total of 315 marine fish were examined, (n=287) were infected. Larval stages (n= 763) encysted within the mesenteries peritoneum and viscera of fish organs were isolated, with a prevalence of 91.11% of infection and, the intensity was 2.65. Molecular analysis was carried out on thirty individuals who have examined the morphology and showed some appearance differences, by amplifying internal transcribed spacers ITS and ITS-1 of nuclear rDNA (rDNA) by PCR using the primer sets NC5/NC2 and SS1/NC13R of thirteen DNA products. Evolutionary analyses were conducted in MEGA X. based on the identity percentage in the GenBank database showed that they belong to anisakid nematodes, in particular, they belong to eleven distinct taxa within the *Hysterothylacium* Ward & Magath, 1917 (Rhabditida, Raphidascardidae) and one identified species *H. amoyense* (Hsü, 1933) Deardorff & Overstreet, 1980. The current study records eleven species that belong to a genus of *Hysterothylacium*; some of the alignment of sequences polymorphisms reveals new different individuals of larvae species that may be adopted as new species if their adult stage is detected, and *N. japonicus* fish considered as a new host record. The current study provides some insights on the systematic taxonomy of these parasites, in addition, it supports similar studies that have been published elsewhere.

Keywords: Anisakid, Arabian Gulf, Molecular characterization, *Nemipterus*, *Japonicus*.

INTRODUCTION

Nemipterus japonicus (Bloch,1791) (Perciformes, Nemiperidae) is commonly known as the Japanese threadfin bream, it is a marine fish native to the Pacific and Indian Oceans (Froese and Pauly, 2021). Nematodes have a worldwide distribution and may include over 1 million species, they are one of the most speciose taxa, with 256 families, and 40,000 species that are known from freshwater, marine, and terrestrial habitats (Anderson, 2000). The richness of the parasitic fauna varies according to the spatial criteria of the presence of the parasite and the host as well as their geographical distribution (Poulin and Morand, 2004). Moreover, the parasite distribution is also impacted by the level of host specificity, which can vary greatly (Krasnovydm *et al.*, 2020).

In the past 30 years, comprehensive studies on marine fish parasites in Iraqi marine waters have been conducted to increase the understanding of the biodiversity of these parasites, based on the morphological structures, which have been limited to the mature stage and are few due to incomplete growth to the adult stage, and because of the abundance of larval stages targeted this group of parasites to show the diversity and genetic variation in the study region. Since there is a bias towards certain groups of parasites, especially towards larger species (Moravec, 2004; Ghadami *et al.*, 2017; Bannai, 2018).

As for studies on fishes of the Arabian Gulf, only a few papers have been previously published. From 1977 to 2013 in different regions of the Arabian Gulf on the coasts of the United Arab Emirates, Qatar, and Iran (Al-Salim *et al.*, 2010). 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 *Hysterothylacium* in Iraqi marine fish, most of these are based on morphology only, providing a limited morphological description that makes specific identification difficult (Ali *et al.*, 2014). However, it needs to be updated to know more about the species and the effect of the environmental changes in regards to the increasing temperature and climate change, these conditions are important to the distribution of this type of parasites (Moravec, 2004).

The recent important advanced studies have been conducted on the same regions, these studies have provided more important and detailed information (Dadar *et al.*, 2016); (Nematollahi *et al.*, 2018) Iran coastal water; WoRMS (2021) listed 90 accepted species of the genus *Hysterothylacium*.

The recoded of these parasite groups are of environmental, medical, and economic importance and therefore agree with the statement which says that this group deserves more research from the perspective of Iraqi biodiversity because knowledge of its parasites is still weak and unfocused, and that they have instigated more in-depth studies in nearby areas and that the integration of their study may provide a full understanding of many of the facts about them in terms of life cycles and their proliferation and there hosts (Spratt, 1997). The lack of information on the genetic diversity structure of the nematode group, which provides a limited morphology, makes it difficult to identify specifically (Ghadami *et al.*, 2017; Bannai, 2018).

The objectives of the present study are to determine the distribution patterns especially the occurrence of parasitic pathogenic infection of humans, as well as their location in the host, provide further information, on the genetic structure, comment on the ascaridoid populations recorded in the current study and compare and discuss the relationship with other closely related taxa in NCBI (2020) databases.

MATERIALS AND METHODS

Description of the study area

The fishing area consists of various locations in Iraqi marine waters, Arabian Gulf (29° 58' 0" 33' 00" N 48° 28' 0" 20" E). This area is inherently different from the rest of the Arabian Gulf, with a diverse hydrodynamic and sedimentary nature due to the presence of many hydrological effects such as the impact of Shatt Al-Arab, Karon River, Shatt Al-Basrah, wave effects, and tidal processes (Albadran, 2004). This area is special, for fish feeding and their breeding. Salinity concentrations in the region from 40 to 43 ppt, water temperature from 12.5 to 33.5 °C.

Specimens collection

A total of 315 *N. japonicus* fish were collected during the period from October 2019 till March 2020, and examined for the prevalence of anisakid nematodes. A variety of methods with various forms of gill nets fishing were used for fish collection. The body cavity and visceral organs were examined, the nematodes were washed extensively in physiological saline (pH 7.4) and stored in 70-95% ethanol at -20 °C for isolation of genomic DNA and PCR amplification. Fish were identified according to Fish Base (Froese and Pauly, 2021). Nematodes were identified using morphology (Shamsi *et al.*, 2013, 2015, 2016; Shamsi, 2016).

Scanning electron microscope

The specimens were fixed in 4 % (v/v) hot formaldehyde solution (60°C), preserved in 70 % (v/v) ethanol, and post-fixed in 1 % osmium tetroxide. The samples were then dehydrated by incubating in a graded series of acetone ethanol concentrations (1:1), (1.5 – 0.5) and absolute acetone, 15 min each (Moravec *et al.*, 2012). A critical-point method was used for sputter-coated with gold (Moravec and Yooyen, 2011).

DNA extraction and Molecular analysis

Genomic DNA was extracted from individual larvae by proteinase K treatment and purified using a mini-column (WizardDNA genomic DNA purification Kit, Promega, USA), according to the manufacturer's protocol. The ITS and ITS-1 of nuclear rDNA (rDNA) were amplified by PCR using the primer sets NC5/NC2 (Forward NC5 (5'-GTA GGT GAA CCT GCG GAA GGA TCA T3') NC2 Revers (5'-TTA GTT TCT TTT CCT CCG CT-3'); and SS1/NC13R ITS-1, Forward SS1 (5- GTT TCC GTA GGT GAA CCT GCG-3), Revers NC13R 5- (GCT GCG TTC TTC ATC GAT -3) (Zhang *et al.*, 2007; Shamsi *et al.*, 2008), respectively, under the same conditions as described previously (Jabbar *et al.*, 2012). The results of the amplification of PCR products were sent to study the sequence in Korea. Sequences were aligned over 1407 positions; the evolutionary history was inferred using the

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Neighbour-Joining method. The ITS sequences determined were compared (using the algorithm BLASTn) with those available in the National Center for Biotechnology Information (NCBI, 2020).

Phylogenetic relationships between characterization diversity of ascaridoid nematodes of *N. japonicus* larvae obtained in the present study and another database of NCBI species. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura & Nei model. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura & Nei model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). Evolutionary analyses were conducted in MEGA X version 10.7.1. (Kumar *et al.*, 2018).

RESULTS AND DISCUSSION

Morphological description

A total of 315, *N. japonicus* (Pls.1, 2) with a total length 176- 215 (195.5) mm, were examined for the detection of nematode parasite: Ascaridoidea (Superfamily), Raphidascarididae (Family), Raphidascaridinae (Subfamily) Raphidascaridinea (Tribe) and *Hysterothylacium* (Genus), from Arabian Gulf (n=287), were infected. Larval stages (n= 763) encysted within the mesenteries peritoneum and viscera of fish organs were isolated, with a prevalence of 91.11% of infection and, the intensity was 2.65.

A total of 247 specimens of larval stages were isolated, with a prevalence of 57.69% of infection. The mean intensity of anisakid nematodes of frequency encountered in this survey was 5.48. Isolated anisakid larvae appeared in the current study under a light microscope cylindrically in shape and are attenuated at both ends, measuring 8-25 mm in length. The anterior extremity of each larva contained an insightful boring tooth that appears distinct in most examined species and four undeveloped labia, that are distinct in most diagnosed species.

The esophagus was characterized by an anterior part with a striated muscle part. A glandular ventriculus is present in most larvae and their measurements varied from one sample examined to another based on the species. The larvae were encysted within the mesenteries peritoneum and viscera of fish organs. Based on morphological characters individuals and the scanning electron micrograph of the cephalic extremity all the individuals were identified morphologically as *Hysterothylacium* with different species (Pl. 2).

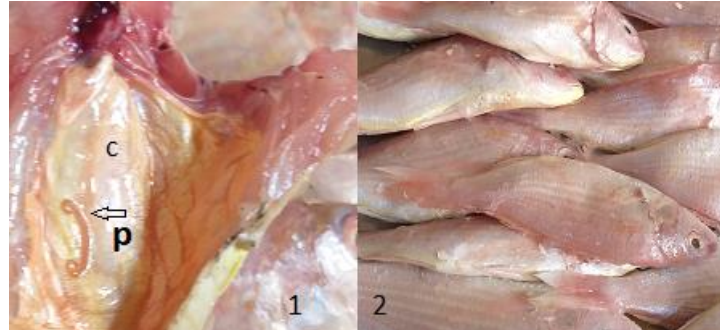


Plate (1): (1): P: larvae (L3) of *Hysterothylacium amoyense*; c: the mesenteries peritoneum and viscera of fish organs, (2) Japanese threadfin bream *N. japonicus* fresh specimens.

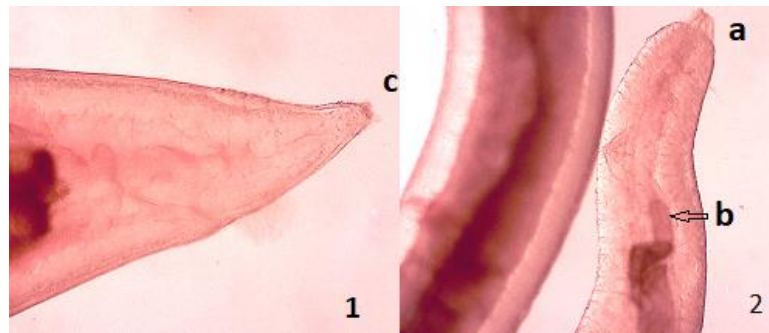


Plate (2): Larvae (L3) of *H. amoyense* viewed under a stereomicroscope; (1) Posterior region of the larval stage morphotypes tail (c), (2) Cephalic region shows mouth opening and papillae (a), glandular ventriculus viewed (b).

Scanning electron microscopy (Pl. 3)

The SEM study revealed a different pattern in the external composition of the cuticle structure. There were different formations in the composition of cuticle folds and longitudinal lateral grooves in the large cuticle among larvae (Plt. 3, 6). The general, mouth opening was surrounded by four small, cephalic papillae (two were dorsolateral and two were ventrolateral), lips not fully developed, and provided by four papillae. It is noted through the general that they belong to different types of parasites and this supports the results of genetic studies as there is a clear differentiation in the order of nitrogen base for each species as described below in Plate (3, 4). Also, the external composition of the cuticle layer confirms the layer of the cuticle, and the appearance of some of the composition of folds in the outer cuticle wall varies by species. The composition of the papillae was completely immature because it was in the larval stage and has not yet matured.

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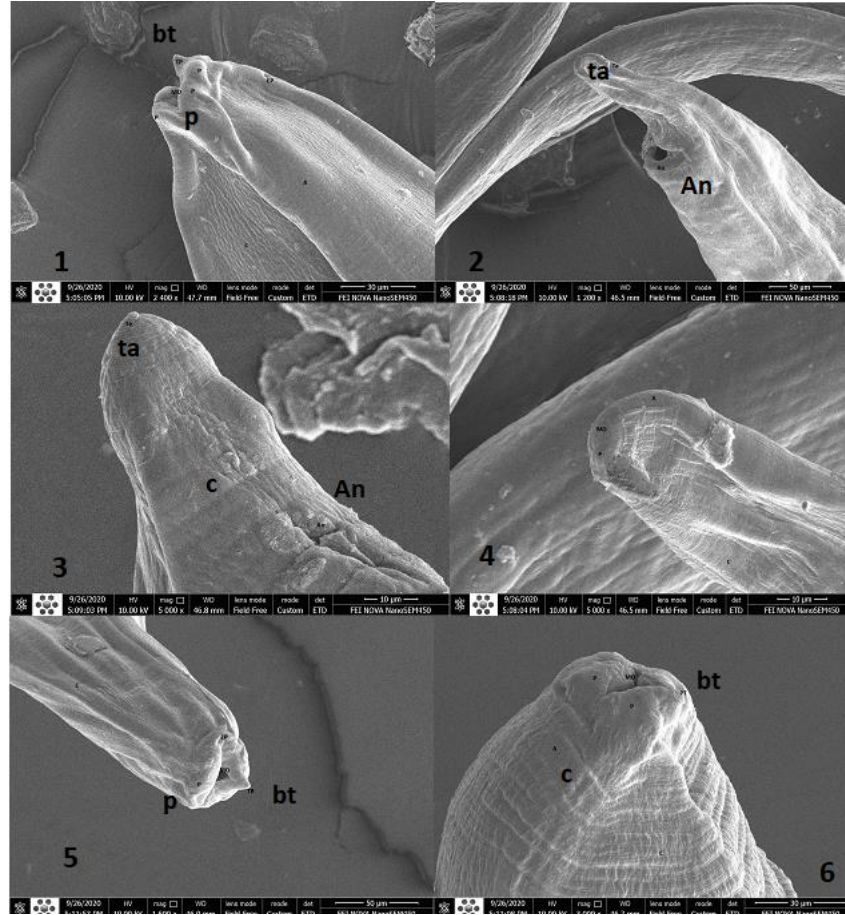


Plate (3): (1) Scanning electron micrograph of the cephalic extremity of the species *Hysterothylacium* sp. 1 larval stage morphotypes, A - Anterior region of the larva, B - a cephalic region of the larva, (C= cuticle of the larva), (bt = boring tooth), (mo = mouth opening). (P= papillae.); (2) Scanning electron micrograph of the posterior region of *Hysterothylacium* sp.1 larval stage morphotypes (TA= tail). (An=anus); (3) Scanning electron micrograph of the posterior region of the species *Hysterothylacium* sp. 2 larval stage morphotypes (TA= tail). (An=anus); (4) Scanning electron micrograph of the cephalic extremity of *Hysterothylacium* sp. 2 larval stage morphotypes, A - Anterior region of the larva, B - a cephalic region of the larva, C - Cuticle of the larva, (cu = cuticle), (mo = mouth opening); (5) Scanning electron micrograph of the cephalic extremity of *Hysterothylacium* sp. 3 larval stage morphotypes, A - Anterior region of the larva, B - a cephalic region of the larva, C - Cuticle of the larva, (p: papilla), (bt = boring tooth); (6) Scanning electron micrograph of the cephalic extremity of *Hysterothylacium* sp. 4 larval stage morphotypes, A - Anterior region of the larva, B - Head region of the larva, C - Cuticle of the larva, (bt = boring tooth). (p: papilla. Bt=boring tooth, cu= cuticle, mo=mouth opening, An=anus, ta=tail).

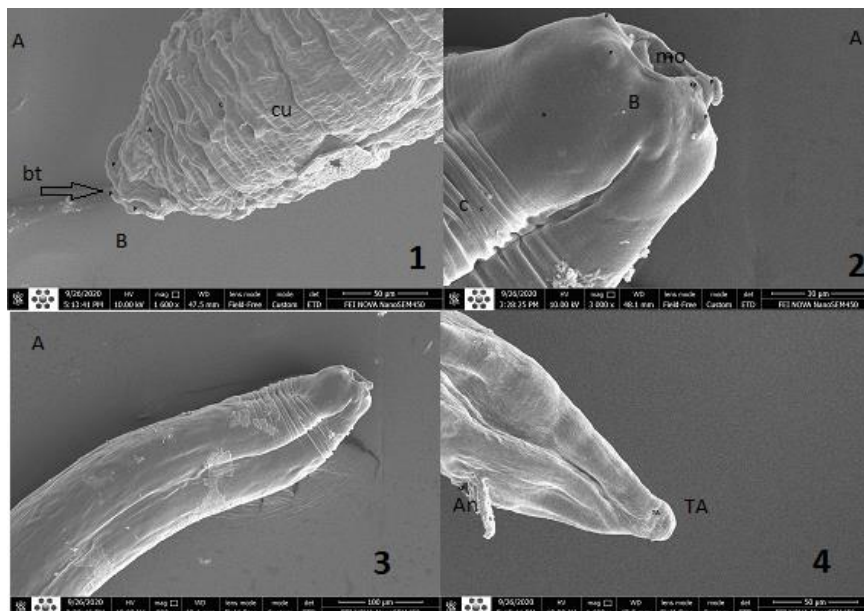


Plate (4): (1) Scanning electron micrograph of the cephalic extremity of *Hysterothylacium* sp. 5 larval stage morphotypes, A- Anterior region of the larva, B – a cephalic region of the larva, (C = Cuticle of the larva), (bt = boring tooth), (cu = cuticle), (mo = mouth opening); (2) Scanning electron micrograph of the cephalic extremity of *Hysterothylacium* sp.6 larval stage morphotypes, A - Anterior region of the larva, B – a cephalic region of the larva, C- Cuticle of the larva (BT = boring tooth), (cu = cuticle), mo = mouth opening; (3) Scanning electron micrograph of the cephalic extremity of *Hysterothylacium* sp.6 larval stage morphotypes. A- Anterior region of the larva, B- a cephalic region of the larva, C- Cuticle of the larva, (bt = boring tooth); (4) Scanning electron micrograph of the posterior region of the species *Hysterothylacium* sp.6 larval stage morphotypes (TA= tail. An=anus). (Bt= boring tooth, cu= cuticle, mo=mouth opening, An=anus, ta=tail).

Molecular identification of anisakid larvae

Molecular analysis was carried out by amplifying internal transcribed spacers (ITS and ITS-1) regions of thirteen individuals. A total of 13 ITS1-5.8S-ITS2 of rDNA gene sequences of the present anisakid larvae from *N. japonicus* (Perciformes, Nemipteridae) from the Arabian Gulf off Iraq. Where deposited in the GenBank under the accession numbers (MW94896, MW961525, MW961524, MW961517, MW961526, MW961520, MW940915, MW961523, MW961527, MW961518, MW961519, MW961522, and MW961521) respectively. while 12 ITS-1 sequences of the product were deposited in the GenBank under the accession numbers (MW961513, MW940880, MW94881, MW940882, MW94883, MW961511, MW961510, MW961509, MW961512, MW961514, MW961515, and MW961516). Detailed information of Alignment of the ITS and ITS-sequence of Ascaridoid nematode species present study with their genetic data including reference source, identical %, and

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GenBank (ITS) reference, and geographical locality. Accession numbers are provided by NCBI for the collected larvae (Tab. 1).

A comparison of the nucleotide sequences of the rDNA of these species revealed low blast scores with the GenBank (percent identity= 97- 100 %). Alignment of the resulting sequences revealed that there was significant variation in the ITS regions, which indicated the presence of different types of larvae. Characterization of the internal transcribed spacers (ITS) of 13 DNA products, based on percentage identities of nucleotides from GenBank, use the online BLAST tool showed the ITS sequences obtained from larvae belong to eleven distinct taxa (*Hysterothylacium* spp.) and two specimens of *H. amoyense*. The amplification has been re-amplified to confirm. Agarose gels analyses revealed for each ITS region amplicons were 1000 – 1100bp. Detailed information of alignment of the ITS sequence of Ascaridoid nematode species present study with their genetic data including reference source, identical %, GenBank (ITS)reference, and geographical locality. Two specimens of *H. amoyense* (MW940915) and (MW940896); query similarity identities percentage was: 922/923(99%) Gaps: 1/923(0% with *H. amoyense* (MT020134.1 Length: 955).

Eleven species of *Hysterothylacium* are recognized, unfortunately, since no mature stages were recorded, it cannot be determined at the species level. The species referred to as *Hysterothylacium* spp. larvae, because haven't been accurately identified to the species level because it needs to study the mature stage of the parasite, using male and female morphology, and characterization of reproductive organs. Detailed information of alignment of the ITS and ITS-1sequence of anisakid nematode species present study with their genetic data including reference source, identical %, GenBank (ITS and ITS-1) reference, and geographical locality. Accession numbers provided by NCBI for the collected larvae are shown in Table (1). The recording of this species is the first in the Arab Gulf region and the world where it did not match it in the sequence in the NCBI. Sequence polymorphisms at alignment related are one to nine different positions of the ITS region, and it revealed as different individuals of *Hysterothylacium* spp. larval type obtained in the present study (Diags. 1-4). It can be considered the *N. japonicus* is a new host record in the world. Besides the most distinguishing characters among *Hysterothylacium* species based on the differences in length and ratio of digestive tracts of nematodes, viz esophagus length, intestinal caecum, appendage and the ratio of each character to each other, it was noted through the follow-up of the sequence of stillness and the different order of nitrogen bases and electron microscope images that there are clear changes among the species diagnosed in the order of the lips and the installation of folds in the outer wall of the parasite.

Table (1): Detailed information of alignment of the ITS and ITS-sequence of ascaridoid nematode species present study with their genetic data including reference source, identical %, GenBank (ITS) reference, and geographical locality. Accession numbers are provided by NCBI for the collected larvae.

Nematode species	GenBank (ITS)	GenBank (ITS-1)	References	Identities %	GenBank (ITS) references	Geographical locality
<i>H. amoyense</i> 17	MW 940896	MW 961513	Guo <i>et al.</i> (2020)	922/923(99%), Gaps: 1/923(0%)	MT020134.1 Length: 955	China
<i>Hysterothylacium</i> sp. 18	MW961525	MW940880	Chen <i>et al.</i> (2018)	925/927(99%), Gaps: 2/927(0%)	MT269312.1 Length: 990	China
<i>Hysterothylacium</i> sp. 19	MW961524	MW94881	Zhang <i>et al.</i> (2018)	882/900(98%), Gaps 9/900(1%)	MH211527.1 Length: 955	China
<i>Hysterothylacium</i> sp. 20	MW961517	MW940882	Zhang <i>et al.</i> (2018)	892/904(99%), Gaps: 7/904 (0%)	MH211518.1 Length: 955	China
<i>Hysterothylacium</i> sp. 21	MW961526	MW94883	Guo <i>et al.</i> (2020)	923/925(99%), Gaps: 1/925(0%)	MT020134.1 Length: 955	China
<i>Hysterothylacium</i> sp. 23	MW961520	MW 99517	Guo <i>et al.</i> (2020)	911/921(99%), Gaps: 4/921(0%)	MT020134.1 Length: 955	China
<i>Hysterothylacium amoyense</i> 24	MW940915	MW961511	Guo <i>et al.</i> (2020)	804/805(99%), Gaps: 1/805(0%)	MF539813.1 Length: 906	China
<i>Hysterothylacium</i> sp. 25	MW961523	MW 961510	Guo <i>et al.</i> (2020)	(916/92899%), Gaps: 6/928(0%)	MT020134.1 Length: 955	China
<i>Hysterothylacium</i> sp. 26	MW96 1527	MW 961509	Guo <i>et al.</i> (2020)	919/926(99%), Gaps: 4/926(0%)	MT020134.1 Length: 955	China
<i>Hysterothylacium</i> sp. 27	MW961518	MW 961512	Guo <i>et al.</i> (2020)	903/929(97%), Gaps8/929 (0%)	MT020134.1 Length: 955	China
<i>Hysterothylacium</i> sp. 28	MW961519	MW 961514	Guo <i>et al.</i> (2020)	916/927(99%), Gaps: 5/927 (0%)	MT020134.1 Length: 955	China
<i>Hysterothylacium</i> sp. 29	MW 961522	MW 961515	Guo <i>et al.</i> (2020)	920/930(99%), Gaps: 8/930 (0%)	MT020134.1 Length: 955	China
<i>Hysterothylacium</i> sp. 30	MW 961521	MW 961516	Guo <i>et al.</i> (2020)	921/929(99%), Gaps: 5/929(0%)	MT020134.1 Length: 955	China

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Query: A17_NC5 Query ID: lcl|Query_442915 Length: 922
Sequence ID: MT020134.1 Length: 955 Range 1: 31 to 953 Score 1698 bits(919), Expect:0.0,
Identities 922/923(99%), Gaps:1/923(0%), Strand: Plus/Plus
Query 1 TCTCCG-
ACGTGCATGCCTTCCATGTGCGCGTATACGTGAGCCGCGCAGCAAGTTGCACA 59
Sbjct 31A..... 90
Query 900 CCCGCTGAATTTAAGCATATAAC 922
Sbjct 931 953
Query: A18_NC5 Query ID: lcl|Query_64135 Length: 927
Sequence ID: MT269312.1 Length: 990 Range 1: 46 to 971 Score 1700 bits (920), Expect:
0.0,
Identities 925/927(99%), Gaps: 2/927(0%), Strand: Plus/Plus
Query 1 TCTCCG-
ACGTGCATGCCTTCCATGTGCGCGTATACGTGAGCCGCGCAGCAAGTTGCACA 59
Sbjct 46A..... 105
Query 900 ACCCGCTGAATTTAAGCATATAACTAA 926
Sbjct 945 971
Query: A19_NC5 Query ID: lcl|Query_46107 Length: 927
Sequence ID: MH211527.1 Length: 955, Range 1: 31 to 922 , Score 1554 bits(841),
Expect:0.0,
Identities 882/900(98%), Gaps 9/900(1%), Strand: Plus/Plus
Query 1 TCTCCG-
ACGTGCATGCCTTCCATGTGCGCGTATACGTGAGCCGCGCAGCAAGTTGCACA 59
Sbjct 31A..... 90
Query 780
GTTGCTCTGTTGGTGGTGTGGTGGTGA AAAAAGGGTTTTGTTGGGATGCATGCTTC
CACG 839
Sbjct 811T.T-...-...-.....A..G.A 866
Query 840
GTTAGTGATTGAGAGTGATGCGAAGGTGGCTATCGCCTCTGTTTGGGACCTCAGG
TCAGT 899
Sbjct 867 .C.....-.....T-.....T.....C..... 922
Query: A20_NC5 Query ID: lcl|Query_12519 Length: 904
Sequence ID: MH211518.1 Length: 955 , Range 1: 41 to 937, Score 1596 bits(864),
Expect:0.0,
Identities 892/904(99%), Gaps: 7/904(0%), Strand: Plus/Plus
Query 1
TGCATGCCTTCCATGTGCGCGTATACGTGAGCCGCGCAGCAAGTTGCACACATGT
GGTGG 60
Sbjct 41 100
Query 781
TGGTGGTGTGGTGGGGATAATGGGGTTGTGTTTGGGAATGCATGCATCAAACAGT
TAGTG 840
Sbjct 821T...-...-.....G-...C..... 873
Query 841
ATGAGAGTGATGCGAGGTGGCTATCGCTCTGTTTTGACCTCAGCTCAGTCGTGAC
TACTC 900
Sbjct 874T.....C. 933
Query 901 GCTG 904
Sbjct 934.... 937
Query: A21_NC5 Query ID: lcl|Query_21131 Length: 926

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Sequence ID: MT020134.1 Length: 955, Range 1: 31 to 955, Score 1696 bits (918), Expect: 0.0,
Identities 923/925(99%), Gaps: 1/925(0%), Strand: Plus/Plus
Query 1 TCTCCG-
ACGTGCATGCCTTCCATGTGCGCGTATACGTGAGCCGCGCAGCAAGTTGCACA 59
Sbjct 31A..... 90
Query 780
GTTGCTCTGTTGGTGCCGTGGTGGTGATATGGTTTGGTGGATGCATGCATCGACA
GCTA 839
Sbjct 811T..... 870
Query 840
GTGATGAGAGTGATGCGAGGTGGCTATCGCTTTGTTTTGACCTCAGCTCAGTCGT
GACTA 899
Sbjct 871 930
Query 900 CCCGCTGAATTTAAGCATATAACTA 924
Sbjct 931 955

Diagram (1): Details sequence polymorphisms pairwise with dots for identifies similarity at the alignment of the ITS sequences representing genotype from the present study, and genotype NCBI blast scores: 1- *H. amoyense* (MW94896), 2- *Hysterothylacium* sp. (MW961525), 3- *Hysterothylacium* sp. (MW961524), 4- *Hysterothylacium* sp. (MW961517), 5- *Hysterothylacium* sp. (MW961526).

Query: A23_NC5 Query ID: lcl|Query_43761 Length: 919
Sequence ID: MT020134.1 Length: 955, Range 1: 31 to 949, Score 1642 bits(889),
Expect:0.0,
Identities 911/921(99%), Gaps:4/921(0%), Strand: Plus/Plus
Query 1
TCTCCGAGCGTGCATGCCTTCCATGTGCGCGTATACGTGAGCCGCGCAGCAAGTT
GCACA 60
Sbjct 31A..... 90
Sbjct 811-..... 868
Query 841 TAGTGATGATAGTGATGCGAGGTGGCTATCGCTTTG-
TTTGACCTCAGCTCAGTCGTGAC 899
Sbjct 869G.....T..... 928
Query 900 TACTGGTGA-TTTAAGAATA 919
Sbjct 929 ...CC.C...A.....C... 949
Query: A24_NC5 Query ID: lcl|Query_472435 Length: 804
Sequence ID: MF539813.1 Length: 906, Range 1: 31 to 835, Score 1480 bits(801), Expect:0.0,
Identities 804/805(99%), Gaps:1/805(0%), Strand: Plus/Plus
Query 1 TCTCCG-
ACGTGCATGCCTTCCATGTGCGCGTATACGTGAGCCGCGCAGCAAGTTGCACA 59
Sbjct 31A..... 90
Query 780 GTTGCTCTGTTGGTGGTGGTGGT 804
Sbjct 811 835
Query: A25_NC5 Query ID: lcl|Query_45659 Length: 927
Sequence ID: MT020134.1 Length: 955, Range 1: 31 to 953, Score:1642 bits(889),
Expect:0.0,
Identities: (916/92899%), Gaps: 6/928(0%), Strand: Plus/Plus

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Query 1 TCTCCG-
 ACGTGCATGCCTTCCATGTGCGCGTATACGTGAGCCGCGCAGCAAGTTGCACA 59
 Sbjct 31A..... 90
 Query 780
 GTTGCTCTGTTGGTGGTGTGGTGGGAAAAAGGGGTTTGGTTTGGGATGCATGCA
 TCGAC 839
 Sbjct 811TG.T.T-...-...-..... 865
 Query 840
 GGTTAGTGATGAGAGTGATGCGAGGTGGCTATCGCTTTGTTTTGACCTCAGCTCA
 GTCGT 899
 Sbjct 866 A.C..... 925
 Query 900 GACTACCCGCTGAATTTAAGCATATAAC 927
 Sbjct 926 953
 Query: A26_NC5 Query ID: lcl|Query_15851 Length: 925
 Sequence ID: MT020134.1 Length: 955, Range 1: 31 to 953, Score 1668 bits(903),
 Expect:0.0,
 Identities 919/926(99%), Gaps: 4/926(0%), Strand: Plus/Plus
 Query 1 TCTCCG-
 ACGTGCATGCCTTCCATGTGCGCGTATACGTGAGCCGCGCAGCAAGTTGCACA 59
 Sbjct 31A..... 90
 Query 780
 GTTGCTCTGTTGGTGGTGTGGTGGTGA AAAAGGGT TTTGGTTTGGATGCATGCATC
 GACGG 839
 Sbjct 811T.T-...-...-.....A. 867
 Query 840
 CTAGTGATGAGAGTGATGCGAGGTGGCTATCGCTTTGTTTTGACCTCAGCTCAGT
 CGTGA 899
 Sbjct 868 927
 Query 900 CTACCCGCTGAATTTAAGCATATAAC 925
 Sbjct 928 953.

Diagram (2): Details sequence polymorphisms pairwise with dots for identifies similarity at the alignment of the ITS sequences representing genotype from the present study, and genotype NCBI blast scores: 1- *Hysterothylacium* sp. (MW961520), 2- *H. amoyense* (MW940915), 3- *Hysterothylacium* sp. (MW961523), 4- *Hysterothylacium* sp. (MW961527), 5- *Hysterothylacium* sp. (MW961518).

Query: A27_NC5 Query ID: lcl|Query_53213 Length: 927
 Sequence ID: MT020134.1 Length: 955, Range 1: 31 to 953, Score: 1565 bits (847), Expect: 0.0,
 Identities 903/929(97%), Gaps: 8/929(0%), Strand: Plus/Plus
 Query 1 TCTCCG-
 ACGTGCATGCCTTCCATGTGCGCGTATACGTGAGCCGCGCAGCAAGTTGCACA 59
 Sbjct 31A..... 90
 Query 780 GTTGCTCTGTTG-
 TGGTGTGGGGGAAAAAGGGGGttttttGGAATGCAGGAAATAAAA 838
 Sbjct 811G.....T..TG.T.T.-...G.....-...T.C.TCG.C. 866
 Query 839
 GGTTATTGATGAAAAGTGATGCAAGGTGGTTATGGCTTTGTTTTGACCTCAGCTC
 AGTCG 898

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Query 1
TCTCCGCGCGTGCATGCCTCTCCATGTGCGCGTATACGTGAGCCGCGCAGCAAGT
TGCAC 60
Sbjct 31AA.....-..... 89
Query 781 TGTGCTCTGTGGTGGTGGTG-
TGAATATGGGTTTGGTTTGGATGCATGCATCGACA 839
Sbjct 810G...-...-...-..... 866
Query 840
GCTAGTGATGAGAGTGATGCGAGGTGGCTATCGCTTGTGTTTTGACCTCAGCTCAG
TCGTG 899
Sbjct 867 926
Query 900 ACTACTCGCTGAATTTAAGCATATAACTA 928
Sbjct 927.....C..... 955

Diagram (4): Details sequence polymorphisms pairwise with dots for identifies similarity at the alignment of the ITS genotype from the present study, and genotype NCBI blast scores of *Hysterothylacium* sp. (MW961521 sequences representing).

Phylogenetic analysis

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. The tree with the highest log likelihood (-9072.97) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with a superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 14 nucleotide sequences. There were a total of 1329 positions in the final dataset. Our results revealed that the *Hysterothylacium* nematodes selected for the phylogenetic tree of 13 gene sequences species constructed with maximum likelihood (ML), were divided into 6 major clades grouped with strong support in clades one, It represents the largest gene diversity of this type of fish, showed that exhibit a very close relationship. whereas other clades represent another diversity of families of the Raphidascarididae (Diag. 5).

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The present study matched one of its aspects with that of a recent study in China by recording two types of nematode parasites, *H. amoyense* (L3), and *Hysterothylacium* sp. (L3). As well as the results of the Guo *et al.*, (2020) study consider the species *H. amoyense* as the most prevalent species were found in the South China Sea. This is exactly what corresponds to the results of the current study if it is found that the *H. amoyense* is prevalent species in *N. japonicus* off Iraq.

Species of *Hysterothylacium* are common nematode parasites of marine fishes worldwide (Guo *et al.*, 2014). Marine fishes can act as both the paratenic/intermediate and/or the definitive hosts of *Hysterothylacium* spp. (Kjøie, 1993). Various studies demonstrated that internal transcribed spacers (ITS, ITS-1, and ITS's-2) of the nuclear ribosomal DNA (rDNA) provide genetic markers for the accurate identification of a range of species of Ascaridoids. Also, more studies indicated that sibling species can be differentiated based on the ITS sequences (Zhou *et al.*, 2002). In the current study, significant changes were observed, The prevalence of some species of parasites that were related to the genus *Anisakis* in the study area has absent when compared to other previous studies in this region, perhaps due to environmental changes, through high temperatures or salinity concentration values, which are important and specific factors for hatching some species of parasites, the rarity of the final host.

The prevalence of infection in intermediate hosts varies greatly, depending on the hosts and age, as well as on the fishing area. This was recorded when compared with studies conducted outside the sampling area in the Arabian Gulf region or open water from the Indian Ocean and adjacent seas. Higher infections with Raphidascarididae were found than that observed by Shamsi *et al.* (2016) in Bander Abas, Hormozan province, off Arabian Gulf. Their study of “600 fish belonging to five popular species of fish. The important studies in Arabian Gulf regions were carried out by Al-Salim and Ali (2010), Ghadami *et al.* (2017), and Zhao *et al.* (2017) from marine fish in Iraqi waters). What they found was that “the *Hysterothylacium* species are perhaps the most abundant and diverse group of marine ascaridoid”. This also corresponds to the results of the current study.

The prevalence of *H. amoyense* in different types of fishes reported by and Chen *et al.* (2018) and Guo *et al.* (2020) that were found in the present study were similar. We also found some distinct morphology and ITS sequences but due to a lack of adult specimens. Unfortunately, we are referred to as *Hysterothylacium* spp. because we haven't accurately identified the species level due to it needs to study the mature stage of the parasite, using male and female morphology, and characterization of reproductive organs.

CONCLUSIONS

Although, the specific broad studies of molecular characterization of ascaridoid nematodes and their larval stages in many parts of the world, the Arabian Gulf nematodes diversity, particularly in Iraqi marine waters, have not been completely understood. Our study provided some insights into the taxonomy and systematics of these parasites, also support similar studies elsewhere both morphological methods and molecular approaches to accurately

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identify large numbers and to determine the abundance, diversity, and infection levels of anisakid nematodes off northwest Arabian Gulf fishery, Iraq, In light of these variables, it has become clear that it is necessary to conduct more cooperation study, with scientific teams in the regions referred to above to study the genetic diversity of ascaridoid to identify its most important species.

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CONFLICT OF INTEREST STATEMENT

We are the authors of the submitting manuscript, declare and confirm that no significant financial or other relationship with any providers of commercial services discussed in the manuscript and any financial supporters of the research.

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التوصيف الجزيئي لأنواع النيوماتودا *Anisakid Nematodes* ضمن جنس
Hysterothylacium من سمكة الباسج الياباني
Nemipterus Japonius (Bloch, 1791) (Perciformes, Nemiperidae)
من المياه البحرية العراقية

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الخلاصة

قدمت هذه الدراسة نظرة جديدة على المعلومات القيمة حول البنية المتنوعة
لسكان الأسكاريدويد وتناقش ثراء الأنواع المحدود في أسماك النوع *Nemipterus japonicus* (Bloch, 1791) رتبة Perciformes، عائلة Nemiperidae.

تتكون منطقة الصيد من مواقع مختلفة في الخليج العربي (29°58' 0 33
E00 20 0 28°48'N)، و تم فحص ما مجموعه 315 سمكة بحرية (n=287) حيث
أصيبت بالعدوى. عزلت مراحل اليرقات (n = 763) المنتشرة داخل الأحشاء من أعضاء
الأسماك ، مع نسبة إصابة 91.11٪ ، وكانت شدة الإصابة 2.65.

أُجري التحليل الجزيئي على ثلاثين فردا من الذين فحصوا مظهريا، التي أظهرت بعض الاختلافات، عن طريق تضخيم منطقة ITS و ITS-1 من rDNA النووية (rDNA) بواسطة PCR باستخدام مجموعات البرايمر التمهيدي NC5/NC2 و SS1/NC13R. وأجريت تحليلات تطويرية في MEGA X؛ واستنادا إلى قاعدة التشابه في البنك الجيني في قاعدة بيانات جينبانك أظهرت أنها تنتمي إلى الديدان الخيطية أنيساكيد، على وجه الخصوص؛ وانها تنتمي إلى أحد عشر صنيفا taxa متميزا من جنس *Hysterothylacium* Ward & Magath, 1917 رتبة Rhabditida، عائلة Raphidascarididae، ونوع واحد تم تحديده متمثلا بالنوع (*H. amoyense* (Hsü, 1933) Deardorff & Overstreet, 1980).

سُجل في الدراسة الحالية أحد عشر نوعا من *Hysterothylacium*، بعض من محاذاة تسلسل تعدد الأشكال، كشفت عن أفراد مختلفين جدد من أنواع اليرقات التي يمكن اعتمادها كنوع جديد، إذا تم الكشف عن الاطوار الكاملة، وان *N. japonicus* هو مضيف جديد في العالم، كما تقدم الدراسة الحالية بعض المفاهيم حول تصنيف هذه الطفيليات، بالإضافة إلى ذلك، تدعم دراسات مماثلة تم نشرها في أماكن أخرى من العالم.