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Infections and molecular characterization of anisakid nematodes from two species of marine fish northwest Arabian gulf

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Abstract

The present study provides new insight into valuable information on the diverse structure of the anisakid population, discusses the limited species richness, and also discusses the relationship with other closely diversity-related taxa in NCBI databases in the *Epinephelus diacanthus* and *Epinephelus coioides* fish. The fishing area consists of various locations in the Arabian Gulf. A total of 69 *E.coioides* and *E. diacanthus* were examined, (n= 48) were infected. Larval stages (n=1,119). Isolated larvae were encysted within the mesenteries peritoneum and viscera of fish organs, with a prevalence of 81.25% of infection and 59.459 % in the *E. diacanthus and E. coioides* respectively. Molecular analysis was carried out on thirty individuals of nematode parasites 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 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 nine distinct taxa within the *Hysterothylacium* spp. The presence of the same species individuals in one host may be the cause of these genetic variations at the species level, and that's what the current study has recorded. It has been found that there is an overlap in the order of nitrogen bases between the same species, and this occurs through the fertilization process, while the rest is clean or have only a few parasites.

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Introduction

Epinephelus diacanthus Spiny cheek grouper, widespread in different parts of the world from the Indian Ocean to Sri Lanka. Previously unknown in areas known to have a wide variety of biodiversity, including the Arabian Gulf and the Red sea, and records in some areas it is known as from the western pacific are based on misidentifications of *Epinephelus stictus* or *Epinephelus fasciatomaculo*, whereas *E. coioides* orange-spotted grouper are more widespread in different parts of the world from the Indo-West Pacific, the Red Sea, South Africa, and Australia, and recently reported from the Mediterranean coast of Israel. Frequently misidentified as *E. tauvina* or *E. malabaricus* (1). 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 (2). Moreover, the parasite distribution is also impacted by the level of host specificity, which can vary greatly (3). As for studies from fishes of the Arabian Gulf, only a few papers have previously been published in the years 1977 to 2013 in different regions of the Arabian Gulf on the coasts of the United Arab Emirates, Qatar, and Iran. 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 (4), they need to update information on species as a result of environmental changes from high temperatures and climate, which are important conditions and determinants of the distribution of this type of parasite

(5). Other studies from fishes of the Arabian Gulf were published. Dadar *et al.* (6) reported the occurrence of Ascaridoidea nematodes from *N. japonicus* in the Arabian Gulf. Nematollahi *et al*. (7) examined 649 *N. japonicus* for helminth parasites in the Arabian Gulf (also off Boushehr, Iran) under the stereomicroscope.The lack of information on the diversity structure of the Nematode group, which provides a limited morphology, makes it difficult to identify specifically (8,9).

The objectives of the present study are to estimate the infection rate and especially the occurrence of parasitic pathogenic infection of humans, as well as their location in the host, provides further information, on the genetic structure. Also, comment on the ascaridoid populations recorded in the current study, compare and discuss the relationship with other closely related taxa in NCBI databases. In addition, that, the following study proved to be the *E. coioides* and *E. diacanthus* are one of the dominant species, and of great economic importance as they are one of the favorite fish in food dishes in this region and it is useful to recognize the diversity of the nematodes parasites in this type of fish.

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’’ N48°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 the Shatt Al-Arab, the Karon River, Shatt Al-Basrah, wave effects, and tidal processes (10). 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 69 *E.coioides* and *E. diacanthus* were 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 under a stereomicroscope, 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 (11).

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 (12). A critical-point method was used for sputter-coated with gold (13).

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 Reverse 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 (14,15), respectively, under the same conditions as described previously . 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 Neighbour-Joining method. The ITS sequences determined were compared (using the algorithm BLASTn) with those available in the National Center for Biotechnology Information (16). NCBI phylogenetic relationships from Alignment, the following criteria were used for comparison Max target sequences 500, max different sequence 0.75, the scale bar indicates the distance in substitution per nucleated. *Anisakis* sp. PNL (MH900217.1) species was used as an outgroup. 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 and 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 and 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). Evolutionary analyses were conducted in MEGA X version 10.7.1 (17).

Results

A total of 69, *E. diacanthus* and *E. coioides* with a total length of 336-470 mm were examined for the detection of ascaridoidea nematode parasites from the Arabian Gulf (n=48) were infected. Larval stages (n= 1119) encysted within the mesenteries peritoneum and viscera of fish organs were isolated (Figure 1), with a prevalence of 81.25 and 59.459% in *E. coioides*. of infection (Table 1).

Isolated anisakid larvae appeared in the current study under a light microscope cylindrically in shape and are attenuated at both ends, measuring 10-25 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. Despite the widespread larval stages and the high intensity of infection, no adult stages were recorded in the fish examined just species of female *philometra* sp., in the orangspotted grouper *E. diacanthus.*

Table 1: Detailed information of Fish species, prevalence, the intensity of infection, and a total of Ascaridoidea nematode collection

Host	Common name	Number	Intensity	Prevalence	Fish infect	Fish exam
E. diacanthus	Orangspotted grouper	683	26.26	81.25	26	32
E. coioides	Spiny cheek grouper	436	19.81	59.459	22	37

The Scanning electron microscopy 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 (Figures 2 and 3).

Figure 1: (1) Orangspotted grouper (*E. diacanthus*) under a stereomicroscope with heavily infected fresh specimens of L3 anisakid (*Hysterothylacium* spp.) larvae viewed. (2) Spiny cheek group from (*E. coioides*) with fresh specimens of L3 anisakid (*Hysterothylacium* spp.) larvae viewed.

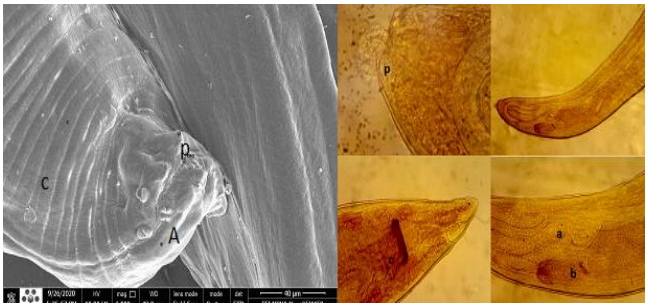

Figure 2: (1) Scanning electron micrograph viewer, the cephalic extremity of the species *Hysterothylacium* sp. (MW422809), larvae collected from *E. coioides*. Larval stage morphotypes A-Anterior and cephalic region of the larva; C-cuticle viewed of the larva. (2) Stereomicroscope viewed different parts of the larvae. p: papillae, a: esophagus, and c: anus.

Molecular analysis was carried out by amplifying internal transcribed spacers ITS and ITS-1 regions of twenty-three individuals. A total of sixteen ITS1-5.8S-ITS2 of rDNA gene sequences of the present anisakid larvae were deposited in the GenBank under the accession numbers MW423787, MW420929, MW411818, MW422807, MW422788, MW422808, MW422169, MW422809, MW422168, MW422166, MW699927, MW423795, MW405344, MW412571, MW422165, and MW423796, respectively, 16 ITS-1 sequences of the product were deposited in the GenBank under the accession numbers MW898637, MW908639, MW901320, MW901252, MW901341, MW901316, MW901351, MW901317, MW898455, MW901318, MW898459, MW901353, MW898579, MW901321, MW901319, and MW928465. 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 and the accession numbers are provided by NCBI for the collected larvae (Tables 2 and 3). Agarose gels analyses revealed for each ITS region amplicons were 1000-1100 bp.

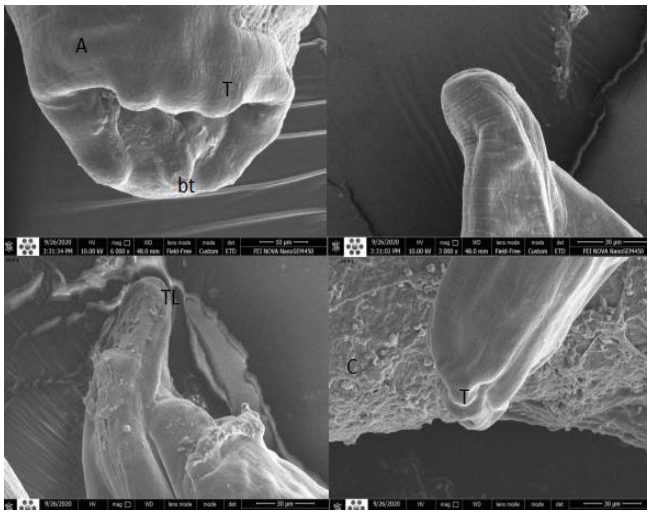

Figure 3: Scanning electron micrograph viewer, of the cephalic extremity of the species *Hysterothylacium* sp (MW699927) of *E. coioides*. The larval stage A: cephalic region of the larva, C- Cuticle of the larva, bt = boring tooth, tl= tail, T= undeveloped labia.

Table 2: Detailed information of present study 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. Accession numbers provided by NCBI for the collected larvae

Nematode species	Fish host	GenBank	Reference	Identical (%)	GenBank references	Country
Hysterothylacium sp.	*E. coioides*	MW423787	24	836/845(99)	KY081888.1	Iran
H. amoyense	*E. coioides*	MW411818	24	837/844(99)	KY081888.1	Iran
H. amoyense	*E. coioides*	MW422807	19	912/913(99)	MT020134.1	China
H. amoyense	*E. coioides*	MW422788	19	917/917(100)	MT020134.1	China
H. amoyense	*E. coioides*	MW422808	19	862/867(99)	MF539813.1	China
H. amoyense	*E. coioides*	MW422169	19	920/926(99)	MT020133.1	China
H. amoyense	*E. coioides*	MW422809	19	915/925(99)	MT020120.1	China
H. amoyense	*E. coioides*	MW422168	19	924/925(99)	MT020111.1	China
C. muraenesoxi	*E. diacanthus*	MW420929	19	910/911(99)	MH211527.1	China
H. amoyense	*E.diacanthus*	MW422166	26	865/868(99)	MF539809.1	China
Hysterothylacium sp.	*E. diacanthus*	MW699927	27	431/434(99)	MH900217.1	India
Hysterothylacium sp.	*E. diacanthus*	MW423795	24	838/846(99)	KY081894.1	Iran
H. amoyense	*E. diacanthus*	MW405344	24	911/916(99)	MT020134.1	Iran
H. amoyense	*E. diacanthus*	MW412571	24	862/869(99)	KT749421.1	Iran
H. amoyense	*E. diacanthus*	MW422165	24	860/873(99)	KT749421.1	Iran
Hysterothylacium sp.	*E. diacanthus*	MW423796	24	802/810(99)	MF539813.1	Iran

Table 3: Detailed information of alignment of the ITS-1 sequence of ascaridoid nematode species present study with their genetic data including reference source, identical %, GenBank (ITS) reference, and geographical locality. Accession numbers provided by NCBI for the collected larvae, GenBank (ITS) references (MH211527.1)

Nematode species	Fish host	GenBank	Identical (%)	Gaps
Hysterothylacium amoyense	*E. diacanthus*	MW898637	458/461(99)	3/461(0%)
Hysterothylacium amoyense	*E. diacanthus*	MW908639	458/459(99%)	0/459(0%)
Hysterothylacium sp	*E. diacanthus*	MW901320	454/457(99%)	1/457(0%)
Hysterothylacium sp	*E. diacanthus*	MW901252	455/459(99%)	4/459(0%)
Hysterothylacium amoyense	*E. diacanthus*	MW901341	455/458(99%)	3/458(0%)
Hysterothylacium sp	*E. diacanthus*	MW901316	456/459(99%)	2/459(0%)
Hysterothylacium sp	*E. diacanthus*	MW901351	452/454(99%)	1/454(0%)
Hysterothylacium sp	*E. diacanthus*	MW901317	470/474(99%)	4/474(0%)
Hysterothylacium amoyense	*E. coioides*	MW898455	456/458(99%)	2/458(0%)
Hysterothylacium sp	*E. coioides*	MW901318	439/453(97%)	5/453(1%)
Hysterothylacium amoyense	*E. coioides*	MW898459	455/458(99%)	2/458(0%)
Hysterothylacium amoyense	*E. coioides*	MW901353	452/454(99%)	0/454(0%)
Hysterothylacium amoyense	*E. coioides*	MW898579	452/454(99%)	0/454(0%)
Hysterothylacium sp	*E. coioides*	MW901321	425/456(93%)	7/456(1%)
Hysterothylacium sp	*E. coioides*	MW901319	455/459(99%)	3/459(0%)
Hysterothylacium sp	*E. coioides*	MW928465	455/458(99%)	3/458(0%)

Characterization of the internal transcribed spacers (ITS) of 16 DNA products, based on percentage identities of nucleotides from GenBank, on used BLAST tool, showed the ITS sequences obtained from larvae belong to sixteen distinct taxa of *Hysterothylacium* spp., with different identities. A comparison of the nucleotide sequences of the rDNA of most species revealed low blast scores with the GenBank (percent identity= 915/925 (99%) and 431/434 (99%) of two nucleotide sequences MW422809 (Figures 4 and 5) and MW699927 (Figure 6), *Hysterothylacium* spp., have not a significant similarity found and low blast scores with the NCBI GenBank database.

Characterization of the internal transcribed spacers of 16 ITS-1 showed that they are belonging seven specie of *Hysterothylacium amoyense* and nine different species of *Hysterothylacium* sp. The alignment of sequence polymorphisms revealed at alignment positions of the ITS - 1 region among the different individuals of *Hysterothylacium* spp., larval type obtained in the present study, of *E. diacanthus* with their genetic data including reference source, identical %, GenBank (ITS) reference with *Hysterothylacium* amoyense isolate 7-6 18S small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed

spacer 2, complete sequence; and 28S large subunit ribosomal RNA gene, partial sequence ID: MH211527.1Length: 955 (Figures 7 and 8).

Figure 4: Alignment of the ITS of *Hysterothylacium* sp. (MW422809), sequences representing genotype from the present study and genotype low blast scores with the *Hysterothylacium amoyense* GenBank (MT020120) pairwise with dots for identifies similarity is identities= 915/925(99%).

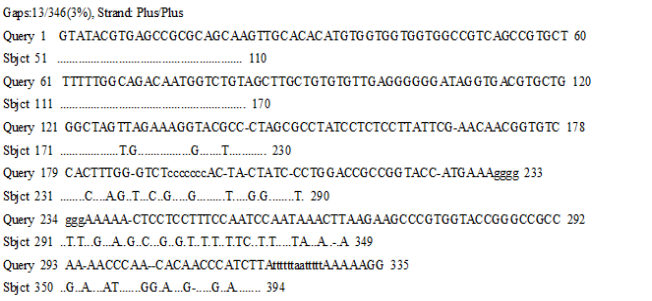

Figure 5: Alignment of the ITS of *Hysterothylacium* sp. (MW699927), sequences representing genotype from the present study, and genotype low blast scores with the *Anisakis* sp. PNLS-550.

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. Based on the identity percentage in the GenBank database showed that they belong to anisakid nematodes, in particular, they belong to nine distinct taxa within the *Hysterothylacium* spp. The presence of the same, species, individuals in one host may be the cause of these genetic variations at the species level, and that's what the current study has recorded. It has been found that there is an overlap in the order of nitrogen bases between the same species, and this occurs through the fertilization process, while the rest is clean or have only a few parasites

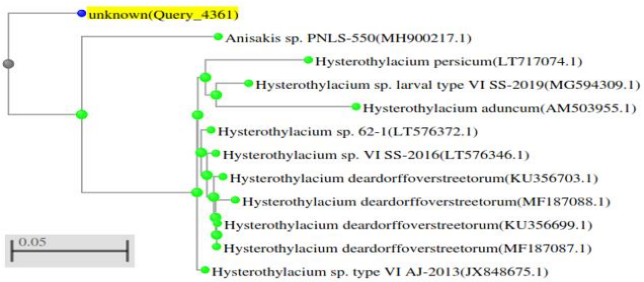

Figure 6: NCBI phylogenetic relationships from Alignment of the ITS of *Hysterothylacium* sp. (MW699927), gene bank data. The following criteria were used for comparison Max target sequences 500, max different seq.0.75, the scale bar indicates the distance in substitution per nucleated. *Anisakis* sp. PNL (MH900217.1) species was used as an outgroup.

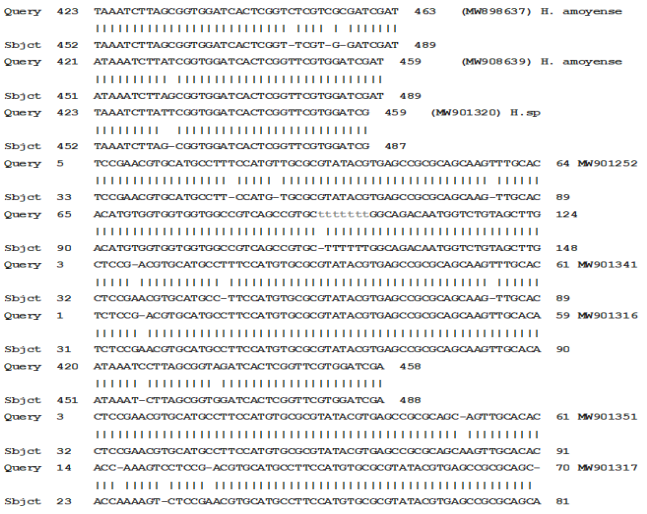

Figure 7: Alignments of sequences polymorphisms revealed at alignment positions of the ITS -1 region among the different individuals of *Hysterothylacium* spp larval type obtained in the present study, of *E. diacanthus* with their genetic data including reference source, identical %, GenBank (ITS) reference with *Hysterothylacium amoyense* partial sequence ID: MH211527.1 Length: 955.

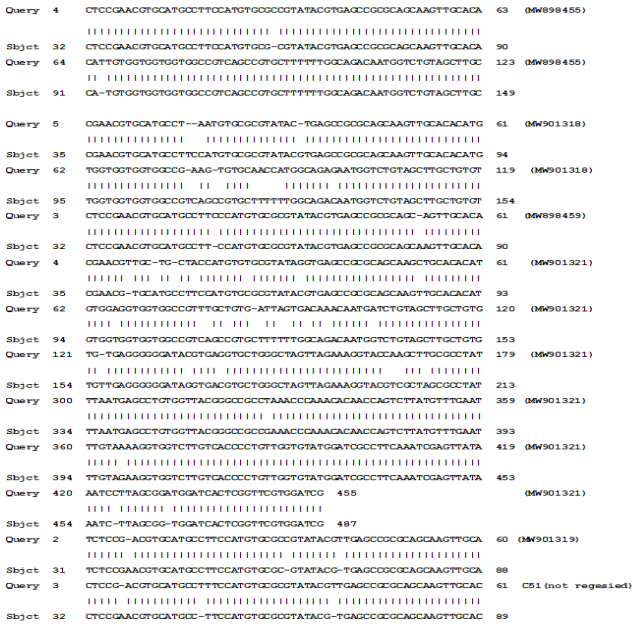

Figure 8: Alignment of sequence polymorphisms revealed at alignment positions of the ITS -1 region among the different individuals of *Hysterothylacium* spp larval type obtained in the present study, of *Epinephelus coioides* with their genetic data including reference source, identical %, GenBank (ITS) reference with *H. amoyense* ID: MH211527.1Length: 955.

Phylogenetic analysis

Our results revealed that the ascaridoid nematodes selected for the phylogenetic tree of 8 gene sequences species constructed with ML, were divided into 4 major clades grouped in the *E. coioides* fish (Figure 9) 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 Clades 2, 3, and 4 represent another diversity. Besides, the phylogenetic tree of 8 gene sequences species constructed was divided also into 3 major clades grouped in the *E. diacanthus* fish (Figure 10) with strong support in clades one, It represents the largest gene diversity of this type of fish, showed that exhibit a very close relationship with 6 species .whereas Clades 2 and 3 represent another diversity of families of the Raphidascarididae. Besides, the evolutionary analysis by maximum likelihood method of the phylogenetic tree of 16 gene sequences species constructed of ITS-1 region was a very close relationship and they were divided also into 4 major clades grouped in the *E. diacanthus* and *E. coioides* fish (Figure 11) with strong support in clades one.

The presence of the same, species, individuals in one host may be the cause of these genetic variations at the species level, and that's what the current study has recorded. It has been found that there is an overlap in the order of nitrogen bases between the same species, and this occurs through the fertilization process, while the rest is clean or have only a few parasites.

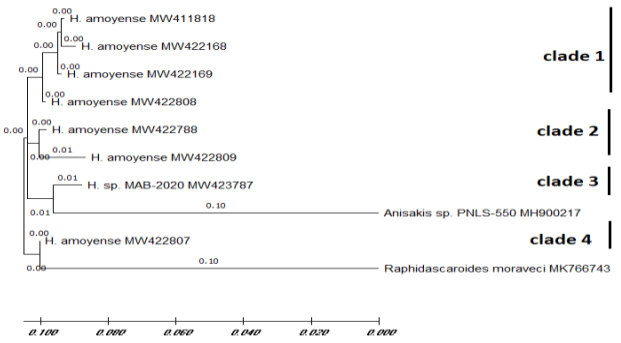

Figure 9: Maximum likelihood (ML) of phylogenetic relationships between characterization diversity of ascaridoid nematodes of *Epinephelus diacanthus* larvae obtained in the present study and another Database of NCBI species. The tree with the highest log likelihood -8651.50 is shown. This analysis involved 10 nucleotide sequences.

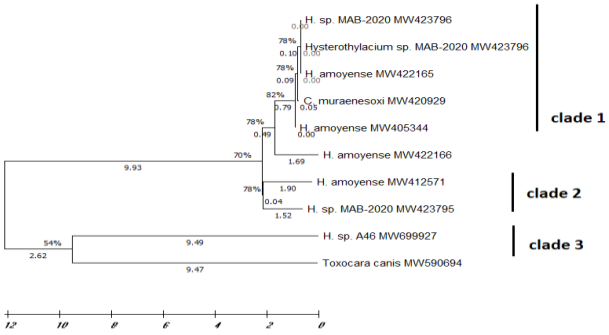

Figure 10: Maximum likelihood (ML) Phylogenetic relationships between characterization diversity of ascaridoid nematodes of *Epinephelus diacanthus* larvae obtained in the present study and another Database of NCBI species. The tree with the highest log likelihood (-8651.50) is shown. This analysis involved 10 nucleotide sequences. There were a total of 1224 positions in the final dataset. *Toxocara canis* (MW 590694) was used as an outgroup.

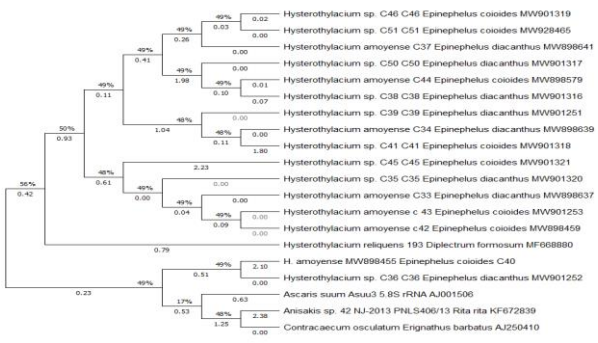

Figure 11: Maximum likelihood (ML) Phylogenetic relationships (ITS-1) of the region between characterization diversity of ascaridoid nematodes of *Epinephelus diacanthus*

and *E.coidided* larvae obtained in the present study and another Database of NCBI species. There were a total of 939 positions in the final dataset. *Ascaris suum* (AJ001506), *Anisaakis* sp (KF672839), and *Contraceacum osculatum* (AJ 250410) were used as an outgroup.

Discussion

Nematodes from the families Anisakidae and Raphidascarididae are commonly referred to as "anisakids, which are known as important pathogens for human and animal health are global parasites where they are widespread and can be found in a variety of different hosts of marine mammals, fish-eating birds, and the most important zoonotic species, most often linked to anisakid nematodes of the genera *Anisakis*, *Contracaecum*, and *Pseudoterranova* (18). The most frequent distribution areas of the Anisakidae family previously reported have been in the Mediterranean region, Japan region, North America, and the North Atlantic Ocean region, since they are fishing areas of economic importance (19). the species diversity diagnosed in the Arabian Gulf is less diverse than the species diagnosed in open water in the oceans, as observed by Shamsi *et al.* (20), this is due to the big difference between the quality of the marine environment in terms of the abundance of intermediate hosts and the different nature of the bottom and the depth of the water, which greatly affects the diversity.

Higher infections with Raphidascarididae were found than that observed by Shamsi *et al*. (20) 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, Iraqi marine water regions were carried out by Al-Salim and Ali (21), Ghadam *et al*. (8), and Zhao *et al.* (22) 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. Species of *Hysterothylacium* are common nematode parasites of marine fishes worldwide (20). This also corresponds to the results of the current study, the significant changes were observed.

Many studies have been conducted on the detection of internal parasites, including larval stages, from these studies carried out by Dadar *et al*. (6) who reported the occurrence of ascaridoid nematodes from *N. japonicus* in the Arabian Gulf. Nematollahi *et al*. (7) examined 649 *N. japonicus* for helminth parasites in the Arabian Gulf (also off Boushehr, Iran). *N. japonicus* is an important marine food fish in Asia. Another important study was carried out by Petter and Sey (23) on the nematode parasites of marine fishes from Kuwait, conducted over 3 years from 1992 to 1995. They suggested a clear convergence in terms of the presence and abundance, and absence of other species belonging to the anisakid family, with the most frequently encountered being anisakid larvae, with eleven different types *Anisakis simplex*, *Terranova* sp. (one type), *Contracaecum* sp. (one type), and *Hysterothylacium* sp. (eight types, KA-KH). This also corresponds to the results of the current study, which shows many similarities with the fauna of (24) in the Arabian Gulf (19,25) in China. 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 (26).

Some of *Hysterothylacium* spp. larvae types were also found in the present study which had identical ITS sequences to those previously reported and identified as *H. amoyense* in the China Sea are consistent with the results of some of the studies of researchers (20,23,26). Since no ITS sequence data from a well-identified number is yet available, we suggest that the assignment of this larval type from the China Sea and the Arabian Gulf to *H. amoyense* is doubtful until future studies on a well-identified specimen of *H. amoyense*. We also found some distinct morphology and ITS sequences of third stages (L3) of unidentified sp., but due to lack of adult specimens, they are referred to as *Hysterothylacium* sp., which may give hypotheses as new species that may need specialized ways to detect them.

Since no mature stages were recorded, it cannot be determined at the species level. 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 8 different positions of the ITS region, and it revealed as different individuals of *Hysterothylacium* larval types that obtained in the present study. It can be considered the *E. diacanthus* and *E. coioides* a new host record For this type of parasite inside and outside the Arabian Gulf region, which is based on a more specialized study to diagnose the species recorded in the current study, the results of which will be reflected in other studies to show the cause of the variation in the species.

Conclusion

In light of these variables, it has become clear that it is necessary to conduct a study in cooperation with scientific teams in the regions referred to above to study the genetic diversity of this genus and identify its most important species. That is why this preliminary survey of this group of parasites should be followed by specialized studies of these species, and by another survey of the groups of parasites belonging to other families. It appears that the nematode fauna of the Arabian Gulf shows many similarities with the fauna of the western Pacific Coast and adjacent seas.

Recently, the accurate identification of ascaridoid larvae to the species level is essential for an evaluation of the molecular epidemiology of the disease. The combination of the sequencing of the ITS region has been widely used for large-scale studies on the identification of ascaridoid larvae to the species level. Also, this study showed the presence of a relatively broad diversity of potentially zoonotic nematodes in edible fish of the Arabian Gulf. Although, their

life cycles and specific identifications of their larval stages in many parts of the world, particularly in Iraqi marine waters, have not been completely understood. Consequently, in the present study, to accurately identify large numbers and to determine the abundance, diversity, and infection levels of anisakid nematodes off northwest Arabian Gulf fishery, Iraq, requires both morphological methods and molecular approaches. Consequently, a future action plan has been prepared to apply some advanced studies to some of the species studied.

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Conflict of interest

No Conflict.

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الإصابات والتوصيف الجزيئي للطفيليات الخيطية من نوعين من الأسماك البحرية من شمال غرب الخليج العربي

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الخلاصة

قدمت هذه الدراسة نظرة جديدة من المعلومات القيمة حول تركيبة مجتمع الطفليات الخيطية في منطقة شمال غرب الخليج العربي وناقشت أثراء الأنواع الموجودة، وأيضا العلاقة مع غيرها من التصنيفات ذات الصلة المتوفرة في قواعد بيانات المركز الوطني لمعلومات التقانة الحياتية في نوعين من اسماك الهامور. تبين من خلال فحص ٦٩ من اسماك الهامور من النوعين *E. coioides* و*E. diacanthus*، ان هنالك ٤٨ سمكة مصابة منها. عزلت الاطوار اليرقية بمراحل مختلفة (ن= ١,١١٩) من احشاء الأسماك، بنسبة إصابة ٨١,٢٥٪ و ٥٩,٤٥٩% من الإصابة الكلية في *E. diacanthus* و *E. coioides* على التوالي. تم إجراء التحليل الجزيئي على ثلاثين فرد من الطفيليات والتي تم فحصها مسبقا وقد اظهرت اختلافا مظهريا واضحا من خلال تضخيم منطقة ITS و ITS-1 من rDNA (rDNA) بواسطة تفاعل السلسلة المتبلمرة باستخدام مجموعات البادئات NC5/NC2 و SS1/NC13R من الحمض النووي المستخلص. وأجريت تحليلات تطورية في برنامج MEGA X. استنادا إلى نسبة التشابة في قاعدة بيانات بنك الجينات أظهرت أنها تنتمي إلى عائلة الطفليات الخيطية، وانها تنتمي إلى تسع فئات متميزة للجنس *Hysterothylacium*. لوحظ ايضا أن هناك تغايرا في ترتيب القواعد النيتروجينية بين مجموعه من الافراد تنتمي لنفس النوع، وتبعا للنتائج المستحصلة اقترحت الدراسة الحالية ان سبب هذه الاختلافات في تسلسل القواعد على مستوى الأنواع ربما يعود الى وجود نفس افراد النوع الواحد في نفس المضيف والتي قد تكون متشابهة مظهريا ومختلفة جينيا و من خلال عملية الإخصاب نتج هذا التغايرات الجينية على مستوى نفس النوع في حين أن الافراد الأخرى احتفظت بنفس التسلسلات الجينية ولم تظهر عليها أي تغيرات جينية.