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RESEARCH PAPER

First record of the nematode *Philometra rischta* parasitizing the testes of *Capoeta trutta* from Lesser Zab River in Kurdistan Region- Iraq.

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A B S T R A C T:

The present study was conducted on the parasitic nematode of the freshwater cyprinid fish *Capoeta trutta* from Lesser Zab River passing Taqtaq District in Kurdistan Region- Iraq. The total 30 fish samples were collected twice monthly from September 2021 until the end of August 2022. They were examined and confirmed the presence of single nematode species belonging to family Philometridae, namely *Philometra rischta* (both gravid and non-gravid females were seen) from abdominal cavity on the testes of *C. trutta*. The collected nematode parasites were examined morphologically under light microscope, morphometric and meristic characters were studied, as well as the molecular study was performed by amplifying and sequencing partial 28S rDNA with primers Forward (F1: 5'-ACCCGCTGAATTTAAGCA-3') and Reverse (R5: 5'-CTCTTCAGAGTACTTTTCAAC-3'). The results showed appearance of *P. rischta* for the first time in Iraq with the prevalence of (6.66%) and mean intensity (1.0).

KEY WORDS: *Philometra rischta*, *Capoeta trutta* Lesser Zab River, morphology, molecular.
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1.INTRODUCTION :

The majority of adult fish parasite nematodes reside in the digestive tracts of the host. Larval stage of nematodes can be detected in every organ, but the mesentery, liver, and musculature are the most prevalent (Molnár *et al*., 2006; Noga, 2010; Gebremedhn and Tsegay 2017).

Numerous species of fish nematode parasites are found in the dracunculoid family Philometridae Baylis & Daubney, 1926. The majority of described species in the 17 philometrid genera that are currently known (Moravec and Shamsi, 2017; Moravec *et al*., 2019) belong to *Philometra*, which comprises parasites that live in freshwater fish body cavities and diverse body tissues (Moravec and de Buron, 2013; Moravec *et al*., 2016).

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The most member of philometrids still little information are known understood about them due to the challenges in studying of these parasites in addition to their unique morphological and biological characteristics (Moravec and Ali, 2014). During recent years, the importance of *Philometra* spp. parasitizing freshwater fishes has increased due to in particular to the rapid development of freshwater aquaculture, because these pathogenic parasites may cause serious damage to infected fishes or, when parasitic in gonads, significantly decrease fish reproduction (Moravec and Ali 2014; Nguyen *et al*., 2021).

Aquatic crustaceans (copepods, ostracods and branchiurids) serve as intermediate hosts in the dracunculoid life cycles, as a result, the transmission and occurrence of these parasites in vertebrate definitive hosts are invariably linked to the aquatic environment. Therefore, it is not surprising that freshwater fishes include the great

majority of dracunculoids life cycle (Periyasamy *et al*., 2020).

Numerous fish nematodes, such as *Philometra* species, have potential harm to their hosts. They can deprive the host of food, enzymes, and vitamins, which can result in mechanical damage, tissue atrophy, obstruction of the alimentary canal, blood arteries, and other ducts, and intoxication from their metabolic products (Molnár *et al*., 2006; Nguyen *et al*., 2021).

The major aim of the current study is to identify the nematodes *Philometra* infecting *Capoeta trutta*, collected from the Lesser Zab River crossing Taqtaq District using morphological (Morphometric and Meristic) and molecular characterization of the collected nematodes in the studied area.

2.MATERIALS AND METHODS

2.1. Study Area

From September 2021 until the end of August 2022, thirty specimens of *Capoeta trutta* were caught by using cast and gill nets twice monthly from the Lesser Zab River in Taqtaq District, which is approximately 90 kilometers southeast of Erbil City. Fish were kept in a cool box with river water as they were alive, transported to the parasitology lab. The fish's abdomen was opened from the ventral side (from the anus to the anterior end), and each organ was separated and examined under a dissecting microscope (Barús and Tenora, 1970). Two female nematodes were isolated (A gravid and non-gravid) and some released larvae of one ruptured gravid female were found, the collected nematodes with the released larvae (except to some of them that transferred directly to double distilled water for molecular examination) were washed in physiological saline, fixed in hot 4% formalin and preserved in cold 4% formalin solution, and cleaned with graduated concentration of ethanol/ glycerin (75/25%; 50/50% and 25/75%) and finally in pure glycerin (1-2 hr. for each concentration) (Moravec and Yooyen, 2011; Moravec *et al*., 2021). Microphotographs were captured using a digital camera (20 megapixels) (Canon power shot G7 X) and drawings were created using a Zeiss picture attachment.

To confirm the identification of the isolated nematode, molecular identification techniques were carried out. The genome of the isolated nematode was extracted from a piece of ruptured female and some released larvae according to the protocol PureLink Genomic kit. The quantity and purity of the extracted genome was ensured by using NanoDrop technique. The extracted genome was amplified using Polymerase Chain Reaction (PCR) and one of the universal primers (28S rDNA) (Forward F1: 5'-ACCCGCTGAATTTAAGCA-3', and Reverse R5: 5'-CTCTTCAGAGTACTTTTCAAC-3'). The sizes of the PCR products were visualized on 1% agarose gel stained with ethidium bromide under UV light for ensuring the sizes of the amplified genes. The PCR products were purified by using EXOSAP-IT (Ambion, CA) prior to bi-directional sequencing. Sanger sequencing were generated at the Oligomer Biotechnology Company-Turkey. The results of the sanger sequencing were analyzed by ChromasPro software (Tamura *et al.*, 2013).

3.RESULTS AND DISCUSSION

A total of 30 *C. trutta* were surveyed for parasitic nematodes during the period of the present study (September of 2021 - end of August 2022). The survey showed the occurrence of *Philometra rischta*. The prevalence and mean intensity of the infections were 6.66% and 1.0 respectively (Table1). The following is a brief account on this parasite.

Table1: fish host exanimated number site of infection, prevalence and mean intensity of *P. rischta*.

zParasite name	Number of fish examined	No. of fish infected	Site of infection	Prevalence %	Mean intensity	Host
Philometra rischta	30	2	testes	6.66	1.0	*Capoeta trutta*

***Philometra rischta* Skryabin, 1923.**

The obtained parasites (two females) were examined morphologically which were cylindrical shapes, covered by smooth cuticle, yellow to light-red color, anus and vulva were missed (Figure 1A; C and 2A; C). Total length of the parasites was 70.9-80.5mm (mean 75.70), width 2.3-2.6 mm. Furthermore, two tiny papillae were found on the mouth of the parasites (Figure 1A; 2A), and the posterior end were rounded shapes that contained two tiny papillae as well but, on lateral view only one papilla appearing (Figure 1D; 2C). Further examined were conducted to know the structures of the obtained parasites, mouth 0.31-0.33 mm (mean 0.32), muscular esophagus 17.2-19.3mm (mean 18.25), muscular gut 21.2-22.7 mm (mean 21.90), glanular intestine 29.2-30.9 (mean 30.05), uterus 29.7-31.6 mm (mean 30.65), nerve ring 19.3-20.6 mm (mean 19.95) from the anterior end (Figure 1A; B; C; D; E, 2A; B; C; D). According to the previous explanation, the obtained gravid female parasites were belonging to the family Philometridae (Molnar and Pazooki, 1995; Moravec and Shamsi, 2017). During isolating the parasites, they were punctured due to the unfavorable environment and thousands of larvae were released (Figure 1C; F, 2 B; E). The length of the larvae was 0.40-0.46 mm (mean 0.43) (Figure 1F, 2E).

The recorded measurements and structures were fitting to that of Moravec (1994) and Tajbakhsh *et al*. (2010) were mentioned nematode *P. rischta* has a muscular esophagus, a smooth cuticle, cephalic papillae and a well-developed esophageal gland. The gravid females have uteri that are yellow, red to crimson in hue and measure 30-42 mm in length (Moravec, 1994; Tajbakhsh *et al*., 2010).

The molecular study of the present samples was done by amplifying and sequencing 28S rDNA partial segment, the results showed amplicons with about 300bp. They were sent to sequencing and showed 98% similarity with that of the *P. rischta* GeneBank sequences according to the sequence ID MH791052.1 (Figure 3; 4).

In additional, Molnár (1966) was mentioned the *P. rischta* collected from *Alburnus alburnus* in Hungary. He described the females of *P. rischta* 35 to 42 mm long, it was found on the inner surface of the gill flap. Males were found only in summer in the swim-bladder serosa; hence, this is the reason why no males noticed in the present study since the time of their presence is too short.

P. rischta found across Europe, as well as in the River Amur and Lake Baikal outside of the continent (Moravec, 1994). Due to their unusual morphology and biology, many species in this family are yet unexplored (undiscovered) or have just limited knowledge (Chabaud, 1975; Moravec, 2004; Roberts, 2021). Lewaschoff (1929), discovered a gravid female of *P. rischta* in the River Volga as late as July. At the present research of the current investigation, mid-April, gravid females with larvae in the uterus were seen, and large numbers of larvae were discovered on the gill cavity and skin of the head. *P. rischta*, for instance, a parasite of the host subcutaneous tissues, is reported from 14 species belonging to 10 genera of Palaearctic freshwater cyprinids (Moravec, 2013).

The molecular study results agreed with that of Montes *et al.* (2022) in which using a female specimen of *P. tahieli* found on the opercular muscle of a juvenile *Micropogonias furnieri* from brackish waters in Argentina using 28S rDNA in their study.

In Iraq, there are a number of studies on fish *Philometra*, according to reports; the first *Philometra* species discovered in Iraqi fish was *P. abdominalis*, which was discovered in the body cavity of an *A. grypus* (syn. *B. grypus*) in the Diyala River (Ali *et al*., 1987). The freshwater fish *C. macrostomum* from Greater Zab River intestine included a *Philometra* sp. larvae (Abubakr, 2015). Fishes from Iraq have produced

10 confirmed *Philometra* species, as well as several unidentified *Philometra* species from different fish host species. The present record of *P. rischta* Skryabin, 1923 in this study regarded as a first one in Iraq since no previous studies mentioned it this country, as well as *C. trutta* regarded as a new host for this parasite.

Moravec and Ali (2014) exclusively described the parasites *P. brachiri* and *P. otolithi* from the ovaries of the tigerteeth croaker *Otolithes ruber* and the oriental sole *Brachirus orientalis*, respectively, both of which were found in Khor Al-Ummaia. Only from the ovaries of the croaker Johnius dussumieri from Khor Al-Ummaia were the ovaries of *P. johnii* described by Moravec and Ali (2013). Additionally, *Epinephelus coioides*, an orange-spotted grouper from Khor Al-Ummaia, was the only fish its ovaries were found to have *P. piscaria* (Moravec and Ali, 2014).

The banded needlefish *Strongylura leiura*, spottail needlefish *S. strongylura*, and hound needlefish *Tylosurus crocodilus* from Khor Al-Ummaia, as well as the hound needlefish *T. crocodilus* from the Al-Fao coast, all were parasitized by two parasite species, *Philometra strongylurae* and *P. tylosuri* (Moravec and Ali, 2005; Ali, 2008). Finally, *Saurida tumbil*, a bigger lizardfish from Khor Al-Ummaia, was used to describe the musculature of *P. tricornuta* (Moravec and Ali, 2014).

Moravec *et al*., (2019) described a new species of the genus *Philometra*, *P. parabrevicollis* (males and subgravid / nongravid females were seen) parasitizing the ovary of the big eye snapper *Lutjanus lutjanus* from the southern coast of Iraq.

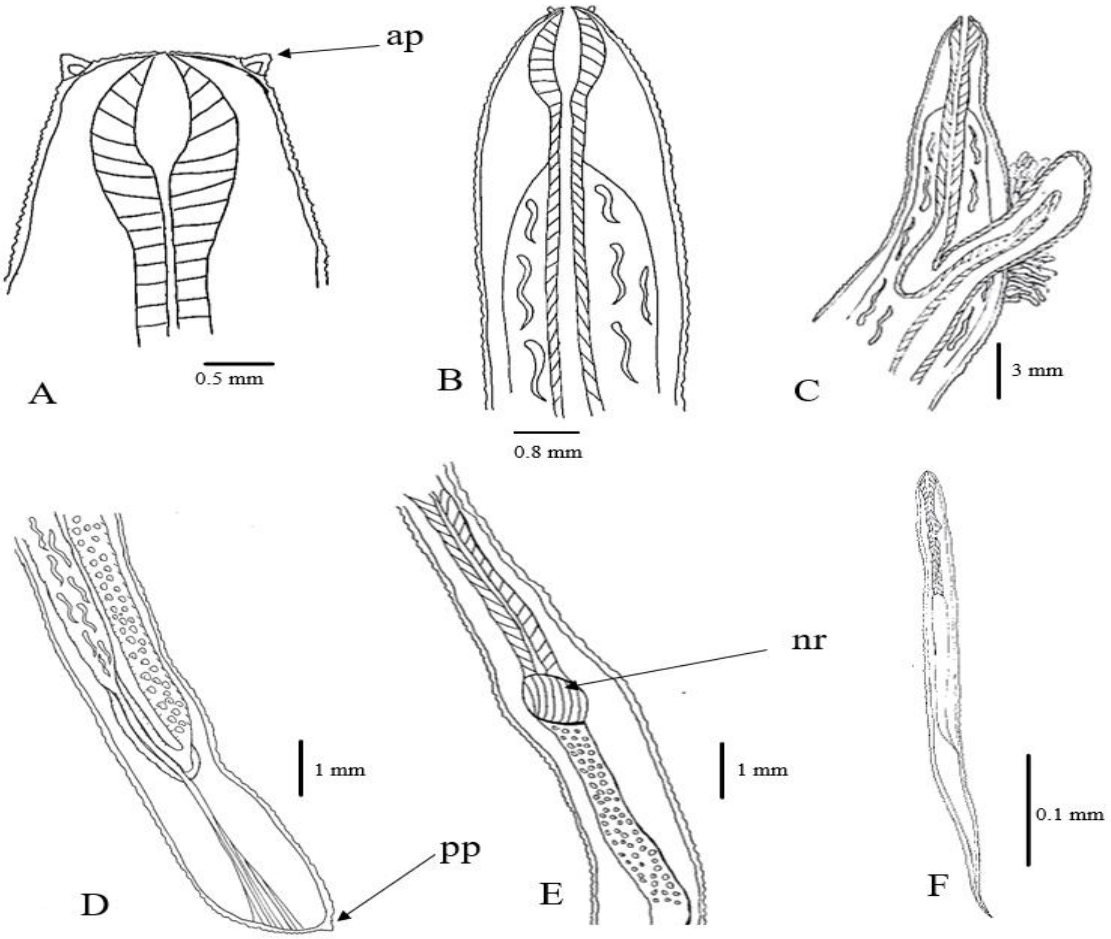

Figure 1: Lucida drawings of female *Philometra rischta*: A- Anterior end, ap- anterior papillae, B- Anterior end, C- Anterior end (rupturing point showing releasing larvae), D- Posterior end, pp- posterior papillae, E- (nr) Nerve ring, F- Larvae.

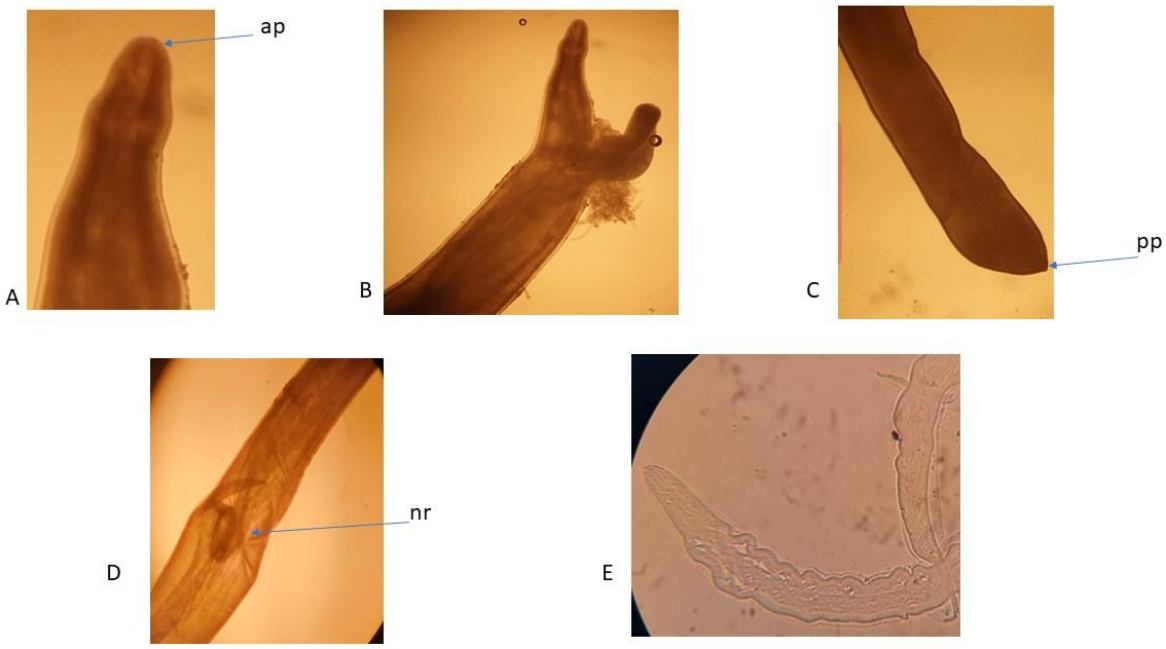

Figure 2: Photomicrograph of female *Philometra rischta*: A- Anterior end (40X), ap- anterior papillae, B- anterior end (40X) (rupturing point showing releasing larvae), C- Posterior end (40X), pp- posterior papillae, D- (nr) Nerve ring (40X), E- Larvae(400X).

Download GenBank Graphics

Philometra rischta isolate Rich large subunit ribosomal RNA gene, partial sequence

Sequence ID: MH791052.1 Length: 1062 Number of Matches: 1

Range 1: 1 to 285 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
486 bits(263)	7e-133	278/285(98%)	1/285(0%)	Plus/Plus

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Query  38   GCTCAGCGCTAAATCTGCTAGTTTTCATAATTAGTCAGAAATGTGGCGTATAGGTGTGAT  97
Sbjct  1    ..C.............GA..C.................................  60

Query  98   TGA-TTTGGATCGTGCGTTTCTCGTTCGCCCTAAGTCCTCCTTGATTGGGGCCACGGTCC  156
Sbjct  61   ...T.........A.....................................  120

Query  157  ATAGAAGGTGCTAGACCTGTTCGGGTGGCGAAACGTACGATACCCGGTCACACTTTAGAG  216
Sbjct  121  ..........................................................  180

Query  217  TCGAGTTGCCTGGGATCGCAGCTCAAAGTAGGTGGTAGACTTCATCTAAAGCTAAATATG  276
Sbjct  181  ..............................................G.........  240

Query  277  GCCACGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAA  321
Sbjct  241  .............................................  285
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Figure 3: Pair-wise alignment of 28S rDNA sequence of *Philometra rischta*. Query is the study or sample sequence and Sbjct is the GenBank sequence.

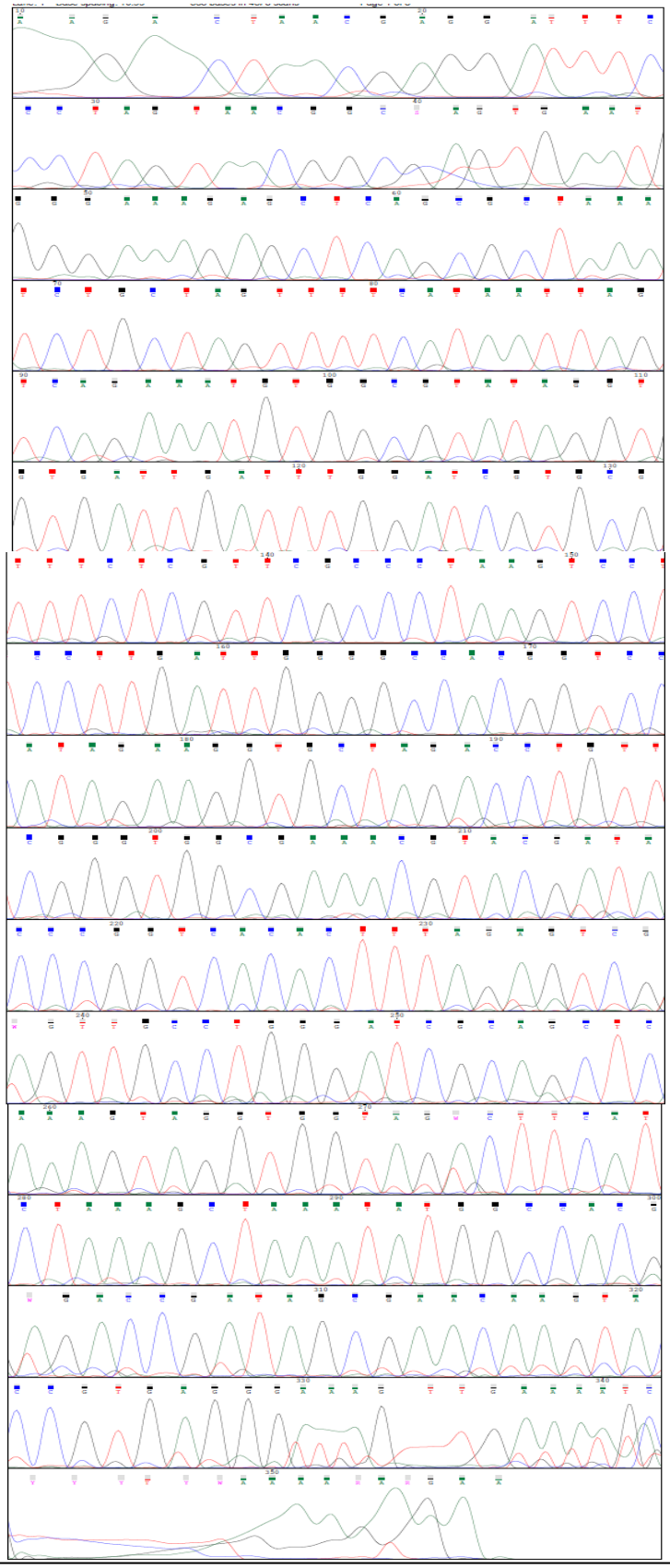

Figure 4: The sequencing result of partial 28S rDNA of *Philometra rischta*.

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