

Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946 Journal homepage: https://ejah.journals.ekb.eg/

Morphometric and molecular study on Capillarids infection in some fresh water fishes Gehan M. Sayed *, Shimaa-Ahmed, M.**, Arafa, M.I.* and Abd-El-Malek, A. M.***

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Received in 2/5/2024 Received in revised from 21/5/2024 Accepted in 25/6/2024

Keywords:

Freshwater fish Capillaria philippinensis larvae Sequence Phylogeny.

ABSTRCT

ipillaria philippinensis larva is a nematode parasite causing intestinal capillariasis, a fish-borne disease that has become more prevalent in the recent years. The present study aimed to investigate the prevalence of Capillaria philippinensis in some fresh water fish in El-Minya Governorate. A total of 110 fish samples [56 wild bajad (Bagrus ba*jad*) and 54 farmed carp fish (*Cyprinus carpio*)] were randomly collected from markets and some fish farms in El-Minya Governorate. In intestinal survey of examined fish, adult nematodes, larvae and high number of free eggs were observed only in intestine of Bagrus bajad, at infection rate of 58.93% (33/56).Regarding morphological characteristics of the detected adult nematodes and their eggs, they were identified as Capillaria spp. The total infection rate of Capillaria spp. in examined fish samples was 30% (33/110). Molecular identification of 25 Capillarid samples isolated from examined fish using recycling PCR technique included sequences and phylogenetic analysis of the Nematode 18S rDNA gene fragment. Confirmed identification of the detected species as Capillaria philippinensis larvae based on the similarity of nucleotide sequences and phylogenetic relationships. In conclusion, this work indicated high prevalence rate of *Capillaria* spp. in wild Bagrus bajad in El-Minya Governorate. Morphological examination of the detected parasite and molecular analysis identified Capillaria philip-

INTRODUCTION

Freshwater fish is considered one of the most important sources of parasitic infection to humans as a result of eating raw or inadequate-

pinensis larvae.

ly cooked small freshwater fish or seafood products, particularly after the increased pollution of rivers and lakes in Egypt (Mohamed, 1996).

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Capillariid nematodes are represented by species in 22 genera, of which nine genera comprise parasites of freshwater, marine and brackish-water teleost fishes (Moravec and Justine, 2010). Only four species described have been found in humans, namely *Capillaria philippinensis, Capillaria plica, Capillaria aerophila*, and *Capillaria hepatica* (Cross, 1992).

C. philippinensis is an important, emerging zoonotic helminth in the recent years which causes intestinal capillariasis that emerged in the 1960s in the Philippines and appeared later causing emerging infection in Egypt (McCarthy and Moore, 2000).

Human infection occurs by ingestion of small freshwater fish, either raw or partially cooked harboring the infective stage larvae (Cross and Belizario, 2007). Larvae are released in the intestine leading to autoinfection, hyperinfection & death if not treated (El-Dib et al. 2015).

In fish; *Capillaria* spp. was detected in carp fish 8.58% at Syria (Salman, 2008), in Myripristis murdjan fish as 22.7% at Saudi Arabia (Khalil et al. 2014). In Egypt, adult *Capillaria* spp. was collected from two species of fresh water fishes; *bagrus bajad* in Dakahlyia Governorate and in *Oreochromis niloticus* (Khalil et al. 2016 & Abdel-Rahman et al. 2019).

Capillaria are commonly found in the intestine of aquarium fish causing ulceration and emaciation. Infested fish with Capillaria showed distended abdomens with pale coloration and poor swimming activity. They appeared to settle in the aquaria bottoms, which ultimately led to a high chance of not being taken for food or predation, lower values of Hb and RBCs count, which may be due to possible passage of *Capillaria sp.* from the stomach to the mesentery leading to possible hemorrhage causing anemia The necrosis of hepatocytes by parasitic infestations, leads to a decreasing of total protein levels in the serum as a result of decreasing protein synthesis, Histological sections of liver, spleen, and intestine of the infested fish showed mild to severe hemorrhage, necrosis, and hyperplasia indicating the mild effect of *Capillaria sp.* parasites in the intestine with a little effect on the visceral organs as liver and spleen. This is may be due to the freely movement inside the intestine and the toxic substances released by the parasite that spread to other tissues (**Nashaat and Maghawri, 2022**).

Most of ornamental fishes like discus (*Symphysodon aequifasciatus*) come from tropical countries. Capillarid nematodes are frequent parasites in both freshwater and marine fishes (Moravec et al. 1988). However, these ornamental fishes are infected with the introduced helminth parasites in aquarium. These helminthes may cause a high mortality in aquarium fishes. For example *Capillaria pterophylli* Heinze, 1933 is known to cause a high mortality in aquarium-kept cichlides (Moravec et al. 1999).

The first human case of capillariasis was detected in Philippines (Chitwood et al. 1964), then in Italy (Chichino et al. 1992), Colombia (Dronda et al. 1993), Korea (Hong et al. 1994) and India (Kang et al. 1994).

In Egypt, human capillariasis were detected by; Youssef et al. (1989) who found the first case in Cairo. Later on in Upper Egypt many cases has been identified and diagnosed {El-Minya Governorate (El-karaksy et al. 2004), Beni-Suef Governorate (Amin, 2011) and Assiut Governorate (Khalifa et al. 2000)}. In Assiut Governorate, five human patients were detected by Khalifa et al. (2000) for the first time, then another case by Abd-El- Rahman (2005) followed by 21 cases (Attia et al. 2012).

This study aimed to highlights screen on prevalence of Capillarids species among wild bajad (*Bagrus bajad*) and farmed carp fish (*Cyprinus carpio*) at El-Minya Governorate, by microscopical and nPCR techniques. Moreover, identify the role of some fish species as suspected as intermediate host for *C. philippinensis* which cause human intestinal capillariasis.

MATERIALS and METHODS

Samples:

A total number of 110 specimens of freshly captured freshwater fish [56 wild bajad (*Bagrus bajad*) and 54 farmed carp fish (*Cyprinus carpio*)] were collected for possible natural infection with *Capillaria* spp. Fish were randomly collected from markets and some fish farms at El-Minya Governorate. The fish samples were transmitted to the laboratory in an ice box.

Parasitological examination:

The stomach and intestine of fish were dissected and placed in separate Petri dishes and washed with physiological saline solution. Fresh smears were done from the sediment of intestinal content and examined microscopically for detection of *Capillaria* eggs (Henriksen and Pohlenz, 1981). Nematodes of alimentary tracts of examined fish were counted and fixed in 70% ethanol; they were cleared using lactophenol and observed under a light microscope, according to Garcia (2001). Identification of the worms to species level was based on their morphological features and dimensions according to **Salman (2008)**. Moreover, different stages were photomicrographed and their measurements were made in micrometers.

Isolated samples of *Capillaria* spp. were collected in Eppendorf tubes containing phosphate buffered saline and washed several times by centrifugation with physiological saline solution. They were kept in 70% ethanol and kept at -70°C for later DNA isolation.

Molecular Studies:

Preparation of specimens of DNA

PCR was performed according to a recommended protocol (Homan et al. 2000). A total of (25) samples were used for recognition of *C. philippinensis* rDNA. Application of PCR for identification of 18S rDNA gene specified for *C. Philippinensis* was performed by using primers supplied from metabion (Germany). They have specific sequence and amplify specific products as shown in Table (1)

Table 1. Oligonucleotide primers sequences of C. philippinensis used for PCR identification system.

Target	Primer sequence (5'-3')	Length of amplified product	Reference		
Nematode 18S rDNA	CGCGAATRGCTCATTACAACAGC	About 900 bp	Floyd <i>et al.</i> , 2005		
	GGGCGGTATCTGATCGCC				

Preparation of PCR Master Mix according to **Emerald** Amp GT PCR mastermix (Takara)

Code No.RR310Akit as shown in Table (2).

Table 2. Preparation of PCR Master Mix.

Component	Volume/reaction	
Emerald Amp GT PCR mastermix (2x premix)	12.5µ1	
PCR grade water	5.5 μ1	
Forward primer(20 pmol)	1 <i>µ1</i>	
Reverse primer (20 pmol)	1 <i>μ1</i>	
Template DNA	5 μ1	
Total	25 μ1	

Cycling conditions of the primers during cPCR

Temperature and time conditions of the primers during PCR are shown in Table (3).

Target	Primary dena- turation	Secondary dena- turation	Annealing	Extension	No. of cycles	Final exten- sion
Nematode	94°C	94°C	54°C	72°C	35	72°C
18S rDNA	5 min.	30 sec.	40 sec.	1 min.		10 min.

Table 3. Cycling conditions of the primers during cPCR

Phylogenetic analysis:

A comparative analysis of sequences was performed using the CLUSTAL W multiple sequence alignment program, version 1.83 of MegAlign module of Lasergene DNA Star software Pair wise, which was designed by **Thompson et al. (1994)** and Phylogenetic analyses were done using maximum likelihood, neighbor joining and maximum parsimony in MEGA6 (Tamura et al. 2013).

Table 4. Prevalence of Capillaria in examined fish.

RESULTS

In the present study *Capillaria* spp. adult and larvae were recovered only in the stomach and intestine of *Bagrus bajad*. The overall infection rate of *Capillaria* in examined fish was 30% (33/110) from all examined fish samples. Different stages of *Capillaria* (adults, eggs and larvae) were recovered only in 33/56 (58.92%) of bajad (*B.bajad*) (Table 4).

l	Bagrus bajad			Carp fish.		Total				
Ex.	Inf.	%	Ex.	Inf.	%	Ex.	Inf.	%		
56	33	58.92	54	0	0	110	33	30		

Parasitological analysis:

Microscopical examination of stomach and intestinal content of examined fish showed all stages of the parasite, *Capillaria* spp. eggs (thin, thick-shelled, embryonated, unembryonated and larvated eggs), larvae (variable sizes) and adults (male& female).

Capillaria female: The adult female appears slender, hair-like, with an envelope-like membrane that covers most of the body surface (Figure 2). The posterior part of the body is rounded and thinner than the anterior part. Its body length ranges from 2.1 to 5.6 mm, and the maximum width at the posterior part of the body is between 58.5 and 84.5 μ m. The anterior part of the body is occupied by esophagus which is formed from a small muscular part followed by a single row of stichocytes closely adhered to each other. The vulva is located near the posterior end of the esophagus (Figure 2&3). The uterus contains barrel-shaped eggs arranged in one row (Figure 5). **Capillaria** male: Its total length is 2.1-3.11mm, and its width is $32.0-48.5\mu$ m, it has a rounded posterior end from which one smooth dorsally curved spicule is extended measuring 68.4μ m (Fig. 1&4).

Eggs: unfertilized, fertilized and larvated *Capillaria* spp. eggs were detected in the present work. Thin-shelled *Capillaria* spp. eggs are brownish in colour, while thick shelled eggs are whitish or grayish in colour. Most *Capillaria* spp. eggs are typically elongated barrel shaped with thick, radially striated shell and flattened bipolar mucoid plugs. They measure 36 to 45 μ m in length by 21 μ m in width (Fig. 6&7).

Larvae: They are cylindrical in shape, bluntly rounded at both ends and vary in size ranging from 1286.5 μ m to1382 μ m. The esophagus occupies more than three quarters of body length. it is very narrow anteriorly and very distinct at its junction with the intestine. The esophagus length is 0.50-0.60 mm (Fig. 8).

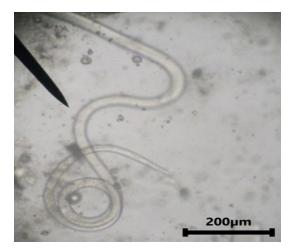


Fig 1. Adult male of Capillaria spp.



Fig 3. Vulval region of an adult female of *Capillaria* spp.



Fig 2. Adult female of *Capillaria* spp. Showing uterus containing eggs

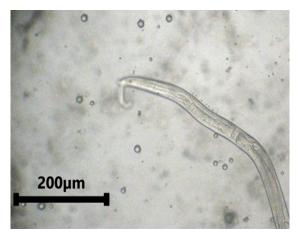


Fig 4. The posterior end of an adult male showing Transparent curved spicule

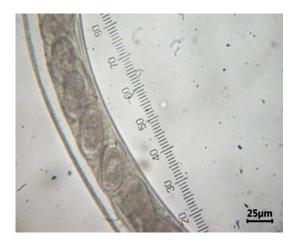


Fig 5. The uterus of *Capillaria* adult female containing peanut eggs

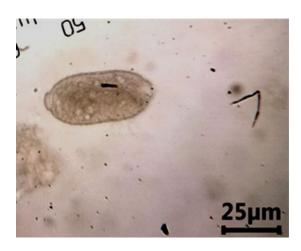


Fig. 6: Thin-shelled *Capillaria* spp. egg with plug- like structure at both ends.

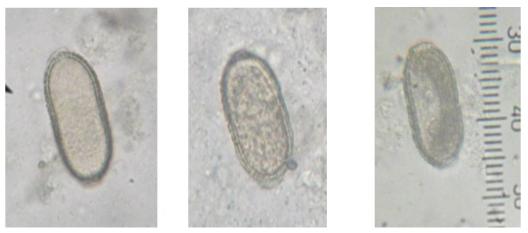


Fig 7. Thick-shelled unfertilized, fertilized and larvated Capillaria spp. eggs x 400.



Fig 8. Capillaria spp. larva x100.

Genotypic study in the present work was essential to detect the zoonotic species among obtained *Capillaria* nematodes as mentioned above.

Genomic DNA (gDNA) was extracted from the ethanol-preserved identified 28 viable

Capillaria sp. adult worms. Amplification of DNA of 18S rDNA gene fragment of *C. philippinensis* was detected in the accurate size and gave one single band which was detected at approximately 900 bp (Fig. 9).

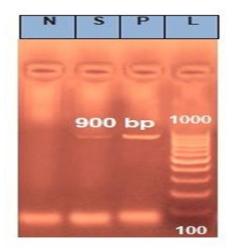


Fig 9. Agarose gel electrophoresis of PCR amplified 18S gene at (900 bp) specific for characterization of *C. philippinen-sis* detected in examined samples.

The expected molecular size of *Capillaria* DNA was detected in all positive *Capillaria* samples by microscopic examination. Partial alignment of the 18S rDNA gene products of *C. philippinensis* produces a sequence of 900 bp has been submitted to the GenBank with the accession numbers OM993296.The obtained sequence was put to BLAST and compared with other available related nematode species sequences from Gene Bank. The BLAST hits result shows that the sequences of the detected isolate are closer to those of *Capillaria sp.* with maximum similarity to *C. philippinensis* (Figure, 10 & 11).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
1		100.0	97.9	98.9	100.0	100.0	100.0	100.0	98.9	98.9	97.9	96.8	91.4	97.9	97.9	94.1	92.5	92.0	92.0	92.0	91.4	91.4	91.4	93.0	93.0	1 KF604920 Par	acapillaria philippinensis
2	0.0		97.9	98.9	100.0	100.0	100.0	100.0	98.9	98.9	97.9	96.8	91.4	97.9	97.9	94.1	92.5	92.0	92.0	92.0	91.4	91.4	91.4	93.0	93.0	2 MZ475992 Par	acapillaria philippinensis
3	2.2	2.2		96.8	97.9	97.9	97.9	97.9	96.8	96.8	95.7	94.7	90.4	95.7	95.7	93.0	93.0	90.9	90.9	90.9	90.4	90.4	90.4	93.0	93.0	3 MW144998 Pa	racapillaria philippinensis
4	1.1	1.1	3.3		98.9	98.9	98.9	98.9	97.9	97.9	96.8	95.7	90.4	96.8	96.8	94.1	92.5	90.9	90.9	90.9	90.4	90.4	90.4	92.0	92.0	4 MW144993 Pa	racapillaria philippinensis
5	0.0	0.0	2.2	1.1		100.0	100.0	100.0	98.9	98.9	97.9	96.8	91.4	97.9	97.9	94.1	92.5	92.0	92.0	92.0	91.4	91.4	91.4	93.0	93.0	5 LC385740 Par	acapillaria philippinensis
6	0.0	0.0	2.2	1.1	0.0		100.0	100.0	98.9	98.9	97.9	96.8	91.4	97.9	97.9	94.1	92.5	92.0	92.0	92.0	91.4	91.4	91.4	93.0	93.0	6 LC385735 Par	acapillaria philippinensis
7	0.0	0.0	2.2	1.1	0.0	0.0		100.0	98.9	98.9	97.9	96.8	91.4	97.9	97.9	94.1	92.5	92.0	92.0	92.0	91.4	91.4	91.4	93.0	93.0	7 LC385734 Par	acapillaria philippinensis
8	0.0	0.0	2.2	1.1	0.0	0.0	0.0		98.9	98.9	97.9	96.8	91.4	97.9	97.9	94.1	92.5	92.0	92.0	92.0	91.4	91.4	91.4	93.0	93.0	8 OM993296 Ca	pillaria philippinensis Sh.M
9	1.1	1.1	3.3	2.2	1.1	1.1	1.1	1.1		100.0	98.9	97.9	92.5	98.9	98.9	95.2	93.6	93.0	93.0	93.0	92.5	92.5	92.5	94.1	94.1	9 LC425004 Bar	uscapillaria obsignata NSMT
10	1.1	1.1	3.3	2.2	1.1	1.1	1.1	1.1	0.0		98.9	97.9	92.5	98.9	98.9	95.2	93.6	93.0	93.0	93.0	92.5	92.5	92.5	94.1	94.1	10 LC052337 Bar	uscapillaria obsignata NSMT
11	2.2	2.2	4.4	3.3	2.2	2.2	2.2	2.2	1.1	1.1		97.9	92.5	98.9	98.9	95.2	93.6	93.0	93.0	93.0	92.5	92.5	91.4	94.1	94.1	11 LC425006 Cap	billaria bursata NSMT:As4480
12	3.3	3.3	5.6	4.4	3.3	3.3	3.3	3.3	2.2	22	2.2		93.6	97.9	97.9	95.2	93.6	93.0	93.0	93.0	93.6	93.6	93.6	93.0	93.0	12 LC052375 Cap	oillaria suis NSMT:As4228
13	9.1	9.1	10.4	10.4	9.1	9.1	9.1	9.1	7.9	7.9	7.9	6.7		93.6	93.6	93.0	93.6	99.5	99.5	99.5	100.0	100.0	98.9	93.0	93.0	13 AY851265 Tric	iuris suis
14	2.2	2.2	4.4	3.3	2.2	2.2	2.2	2.2	1.1	1.1	1.1	2.2	6.7		100.0	96.3	94.7	93.0	93.0	93.0	93.6	93.6	92.5	95.2	95.2	14 LC052365 Aon	chotheca putorii
15	2.2	2.2	4.4	3.3	2.2	2.2	2.2	2.2	1.1	1.1	1.1	2.2	6.7	0.0		96.3	94.7	93.0	93.0	93.0	93.6	93.6	92.5	95.2	95.2	15 LC052364 Aon	chotheca putorii NSMT:As426
16	6.1	6.1	7.4	6.2	6.1	6.1	6.1	6.1	5.0	5.0	5.0	5.0	7.3	3.8	3.8		98.4	92.5	92.5	92.5	93.0	93.0	92.0	91.4	91.4	16 MN148644 Ca	pillaria madseni ISTUC1_2019
17	7.9	7.9	7.3	7.9	7.9	7.9	7.9	7.9	6.7	6.7	6.7	6.7	6.7	5.5	5.5	1.6		93.0	93.0	93.0	93.6	93.6	92.5	91.4	91.4	17 LC052348 Cap	oillaria madseni NSMT:As4069
18	8.5	8.5	9.7	9.7	8.5	8.5	8.5	8.5	7.3	7.3	7.3	7.3	0.5	7.3	7.3	7.9	7.3		100.0	100.0	99.5	99.5	98.4	92.5	92.5	18 HF586913 Tric	huris leporis TI1
19	8.5	8.5	9.7	9.7	8.5	8.5	8.5	8.5	7.3	7.3	7.3	7.3	0.5	7.3	7.3	7.9	7.3	0.0		100.0	99.5	99.5	98.4	92.5	92.5	19 HF586912 Tric	huris skrjabini Tsk1
20	8.5	8.5	9.7	9.7	8.5	8.5	8.5	8.5	7.3	7.3	7.3	7.3	0.5	7.3	7.3	7.9	7.3	0.0	0.0		99.5	99.5	98.4	92.5	92.5	20 HF586911 Tric	huris ovis To1
21	9.1	9.1	10.4	10.4	9.1	9.1	9.1	9.1	7.9	7.9	7.9	6.7	0.0	6.7	6.7	7.3	6.7	0.5	0.5	0.5		100.0	98.9	93.0	93.0	21 HF586909 Tric	huris vulpis Tv1
22	9.1	9.1	10.4	10.4	9.1	9.1	9.1	9.1	7.9	7.9	7.9	6.7	0.0	6.7	6.7	7.3	6.7	0.5	0.5	0.5	0.0		98.9	93.0	93.0	22 HF586907 Tric	huris muris Tm1
23	9.1	9.1	10.3	10.3	9.1	9.1	9.1	9.1	7.9	7.9	9.1	6.7	1.1	7.9	7.9	8.5	7.9	1.6	1.6	1.6	1.1	1.1			92.0		churis trichiura TST332
24	7.4	7.4	7.4	8.7	7.4	7.4	7.4	7.4	6.3	6.3	6.2	7.4	7.3	5.1	5.1	9.2	9.1	7.9	7.9	7.9	7.3	7.3	8.5		100.0	24 KJ579684 Cap	illaria aerophila CZ2CAR_D
25	7.4	7.4	7.4	8.7	7.4	7.4	7.4	7.4	6.3	6.3	6.2	7.4	7.3	5.1	5.1	9.2	9.1	7.9	7.9	7.9	7.3	7.3	8.5	0.0		25 KJ579683 Cap	illaria aerophila CZ2CAR_C
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		

Fig 10. Partial alignment of the 18S rDNA gene of *C. philippinensis* with related species (GenBank accession numbers are indicated after species name). Sequence distance of the 18S rRNA gene of the tested nematode strain (generated by lasergene software) showing identity range of 97.9%- 100% with *paracapillaria philippinensis* strain

KF604920 P. philippinensis	20 20 20 40 50 60 70 80 90 100 120 120 120 140 150 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 170 140 140 140 140 140 140 140 140 140 14
MZ475992 P. philippinensis OM993296 C. philippinensis Sh. LC425004 B. obsignata	Δ
LC425006 C. bursata LC052375 C. suis	CA
AY851265 T. suis LC052365 A. putorii	
MW148644 C. madsenn HF586913 T. leporis HF586911 T. ovis	
KJ579684 C. aerophila	

Fig 11. Nucleotide alignment report for all studied strains. Nucleotide alignment of the analyzed strains showing great homology among the current study strains and *Paracapillaria philippinensis* (Accessions KF604920 and MZ475992).

Phylogenetic tree shows the evolutionary relationship of the sequences in which the length of the horizontal line was proportional to the estimated genetic distance between the sequences. Such tree indicated that the evolutionary distance between groups is very short. (Fig.12) show in great homology between sequences.

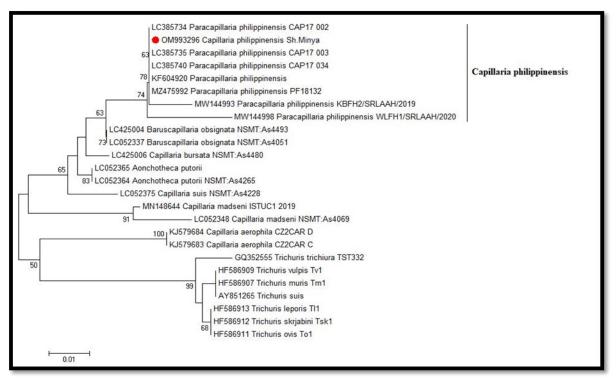


Fig 12. Phylogenetic analysis of the 18S rDNA gene of *C. philippinensis* larvae sequence (GenBank accession number OM993296) with related species (GenBank accession numbers are indicated after species name).

DISCUSSION

Human intestinal capillariasis is caused by *C. philippinensis* larvae and considered an important public health problem that may lead to death in cases of improper treatment. Small freshwater and brackish-water fish are the source of infection and probably fish-eating birds, the reservoir host (**Cross, 1998**).

The parasite has a unique life cycle. Experimental studies revealed the life cycle of the parasite and the mode of infection as a result of ingestion of raw fresh or brackish-water fish containing infective larvae in their intestine (Cross and Basaca-Sevilla, 1986).Small fish are mostly eaten raw, as they are mostly not eviscerated; so, if it is infected, the production of thick shelled eggs and infective larvae is alternating in the same host (Bair et al. 2004, Lu et al. 2006). Recently in Egypt, human intestinal capillariasis has increased, indicating that we have a natural intermediate host, which is mostly freshwater fish.

In the present work, both *Bagrus bajad* (wild fish) and carp (cultured fish) were examined to reveal the source of *C. philippinensis* infection in Upper Egypt. Infection with *Capillaria* spp. was recovered only in bajad (*Bagrus bajad*) and was not detected in carp fish. Many authors detected adult or larval stage of *Capillaria* spp. from intestine of *Bagrus bajad* (Mohamed et al. 2003) and from the stomach of the fresh water fishes, *B. bajad* (Khalil et al. 2016).

The overall infection rate of *Capillaria* in examined fish was 33% from all examined fish samples, which only detected in Bajad species with 58.92%. The present result is considered

higher than those reported by **Thilakaratne et al. (2003)** in Siri Lanka which was 4%. **Khalil et al. (2014)** recorded that the prevalence of *Capillaria* spp. was 15 % in Bajad collected from fish in southern Saudi Arabia. In Nigeria, **Okpasuo et al. (2016)** reported that the infectivity rate of *Capillaria* spp. in Bargus bajad was 25%.

On the other hand, the results of the present study are considered lower than those reported by **Shehzad and Ansari (2018)** and **Abdel-Rahman et al. (2019)** who determined the *Capillaria* infection in all examined Bajad samples (100%). The present and previous data cleared that Bajad fish is the main host (final or intermediate) for *Capillaria* spp. in examined areas.

In the current study, *Capillaria* spp. is detected only in wild fish (Bajad) 58% and not detected in cultured fish (carp fish). This result agree with **Abdel-Rahman et al. (2019)** who recorded *Capillaria* in nearly all collected wild fresh water, *B. bajad.*

The highest infection rate of wild fish with *Capillaria* spp. may be attributed to various factors including the locality from which fish were caught, frequent contamination of fresh water with human excreta and migratory fish eating birds which act as reservoir hosts **(Cross, 1998).** So, it is important to avoid defection in water resources, proper cooking of fish, thorough washing of hands, wearing disposable gloves during processing of fish to prevent infection with *Capillaria* (**Ziarati et al. 2022**).

Morphologically, different stages of nematode helminthes recovered in the present work were identified as *Capillaria* spp. Stages of *Capillaria* spp. were detected previously either in human being or fish by Youssef et al. (1989), Moravec (2001), El-karaksy et al. (2004), Abd-Elsalam et al. (2012) Attia et al. (2012) and Abdel-Rahman et al.(2019).

In spite of the recovered morphological characteristics of different stages of *Capillaria* spp. had previously been reported, we redescribed some of their characteristic features to

beuseful for diagnosis and identification of *Capillaria* spp.

As the available literature about genetic identification of *C. philippinensis* of fish were limited, for which, the current study is one of the earliest studies to detect *C. philippinensis* infection of fish.

Diagnosis of *Capillaria* using nPCR targeting cap 18s gene offers a relatively acceptable method for diagnosis and determination of the true prevalence of infection. It can also help the microscopical diagnostic tools of *Capillaria philippinensis* zoonotic infection (Floyd et al. 2002). The primers were reported to be specific and sensitive to *Capillaria*-DNA because the amplified region of the ssurDNA is highly conserved for *C. philippinensis* (El-Dib et al. 2015).

In the present study, recycling PCR was performed to confirm C. philippinensis larvae infection among fish cases diagnosed by microscopy. Our study reported that twenty five samples of the positive cases using microscopic examination were positive for C. philippinensis larvae via PCR technique. Nucleotide alignment of the analyzed strain showed great homology with paracapillaria philippinensis (Accessions KF604920 and MZ475992). Ali et al. (2016) reported that the detection of capillariasis using nested PCR is a specific and accurate method that helps to determine the true prevalence and epidemiology of the disease, they considered the nested PCR as a gold standard test for diagnosis of Capillaria infection.

On the other hand, the present study confirms what was indicated by **Abdel-Rahman** et al. (2019) about the usefulness of using fish *Capillaria* antigen in diagnosis of *C. philippinensis* in human infection.

In conclusion, the present work indicated high prevalence rate of *C. philippinensis* infection in wild *Bagrus bajad*. Both morphological examination of the parasite and molecular analysis confirmed identification of *C. philippinensis* larvae in examined fish.

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