Annals of Parasitology 2019, 65(4), 365–370
doi: 10.17420/ap6504.222

Original papers

The first record of *Ascocotyle* (*Phagicola*) *longa* (Digenea: Heterophyidae) metacercariae as the fish-borne zoonotic trematode in *Chelon auratus* and *Chelon saliens* (Pisces: Mugilidae) around the cage culture in the Caspian Sea, Iran

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ABSTRACT. As a matter of fact, there has not been any reportage of the parasite of *Ascocotyle* (*Phagicola*) *longa* Ransom, 1920 from the Caspian Sea, Iran. During two years, April 2017 until 2018, 158 specimens of mullet fish, *Chelon auratus* and *Chelon saliens* (Risso, 1810) were collected and carefully examined considering the standard parasitological methods. Interestingly, the metacercaria was found in the stomach and intestine of the mullets. Morphological and morphometric analyses indicated that this parasite species belongs to *Ascoctyle* (*Phagicola*) *longa*. Overall infection mean intensity (52.1±128.3), mean abundance (14.8±71.9) and prevalence (28.5%) were calculated in mullets. Differences in the infection level of *Ascocotyle* (*Phagicola*) *longa* in relation to fish species, location and sampling years are determined and discussed. So that the mean intensity of this parasite is significantly different in the two locations of Anzali and Kiashahr (X^2=13.35, df=1, p=0.00<0.05) and the mean intensity between mullet species (X^2=5.49, df=1, p=0.019<0.05) is significantly different. In fact, *A.* (*Phagicola*) *longa* is a widespread and rife fish-borne zoonotic parasite and also one of the most notable agents of human heterophyosis observed in the Caspian Sea.

Keywords: Heterophyidae, metacercariae, stomach, intestine, Mugilidae, Caspian Sea

Introduction

Heterophyid is a type of food-borne zoonotic trematodes with a length of 1–2 mm and has at least 36 genera [1] while 13 genera of them are known as zoonotic parasites [2]. Besides, *Ascocotyle* (*Phagicola*) *longa*, as a fish-borne zoonotic trematode, has a vast and wide global distribution [3].

Actually, aquatic gastropods and fish, especially mullets, are the first and second intermediate hosts of this parasite, while, mammals and fish-eating birds are the final hosts [4,5]. Meanwhile, the metacercariae of these parasite are encysted in the tissues of the gills, heart, mesenteries, muscles, liver, intestine, stomach wall, gall bladder, gonads, brain, spleen and kidney of the mullet fish [6–9].

According to the scientific reports, *Ascocotyle* (*Phagicola*) *longa* is mostly found in the mullets, *Mugil platanus* [6], *M. liza* [7,9], *M. incilis* [10], *M. cephalus* [11], *Liza aurata* [12] and other several fishes for example *Gobiesox fluviatilis* (Cyprinodontidae); *Dormitator latifrons*, *D. maculatus* (Eleotridae); *Centropomus robalito* (Centropomidae); *Cichlasoma trimaculatum* (Cichlidae); *Symphurus nigrescens* (Cynoglossidae); *Astyanax fasciatus* (Characidae) and *Poecilia sphenops* (Poeciliidae) [8,13,14].

Assessing some practical aspects of this study we have to highlight that, there are three species of the mugilids fish including *Chelon saliens* and *C. auratus* in the Caspian Sea and *Planiliza abu* in the southern fresh waters, which are known as the main notable commercial species [15,16] and are often consumed fresh, smoked or canned. Besides, it is also used as a bait and as cultured fish in fish farming [15]. Furthermore, the infected mullet fish can lead to heterophyiasis disease in humans and it can be a remarkable problem for the public health. As the final point, the aim of this discussion is about investigating the infestation rate of mullets around

Table 1. Morphometrics (in µm and ranges in parentheses) excysted metacercaria in present study in Iran (N= 0) and comparison with Simões et al. [9], Martorelli et al. [17] and Santos et al. [7] research on *Ascocotyle* (*Phagicola*) *longa* isolated from internal organs in *Mugil liza*

Character	Present study	Simões et al. [9]	Martorelli et al.[17]	Santos et al. [7]
Cyst length	311 (253–372)	232 (225–240)	290 (210–425)	–
Cyst width	218 (214–261)	229 (215–235)	253 (195–300)	–
Body length	504 (483–653)	443 (400–490)	478 (384–600)	450 (380–590)
Body width	212 (201–318)	142 (120–150)	94 (52–202)	120 (100–160)
Oral sucker length	40 (24–55)	26 (20–30)	22 (13–45)	36 (23–53)
Oral sucker width	27 (21–39)	48 (40–50)	21 (12–52)	37 (20–48)
Ventral sucker length	34 (24–46)	30 (25–35)	39 (28–75)	29 (23–38)
Ventral sucker width	25 (20–34)	30 (25–35)	38 (24–80)	34 (25–45)
Spine length	12 (11–15)	–	–	–
Spine width	3 (3–4)	–	–	–
Circumoral spines	16 (15–18)	15 (13–18)	16 (14–17)	14 (15–18)

cages to the zoonotic parasite.

Materials and Methods

A total of 158 mullets were collected from two coasts of Anzali (37°28′19.29″N, 49°30′52.26″E) and Kiashahr (37°24′10.49″N, 50°04′46.21″E) near the fish cage culture on the southern parts of the Caspian Sea. The sampling process was performed from April 2017 till 2018. It is noteworthy to say that, the biometric parameters were done in the laboratory, carefully. In the next step, the parasites of each fish were evaluated considering the standard methods. The number of parasites and the site of infection was recorded, as well. The isolated helminths were stored in 70% ethanol or 10% formalin solution and mounted on permanent microscope slides with Canada balsam. We used the software AxioVision 4.5 to conduct the morphometry stage. Then the samples were identified using Simões et al. [9], Scholz [3], Scholz et al. [8], Martorelli et al. [17]. For scanning electron

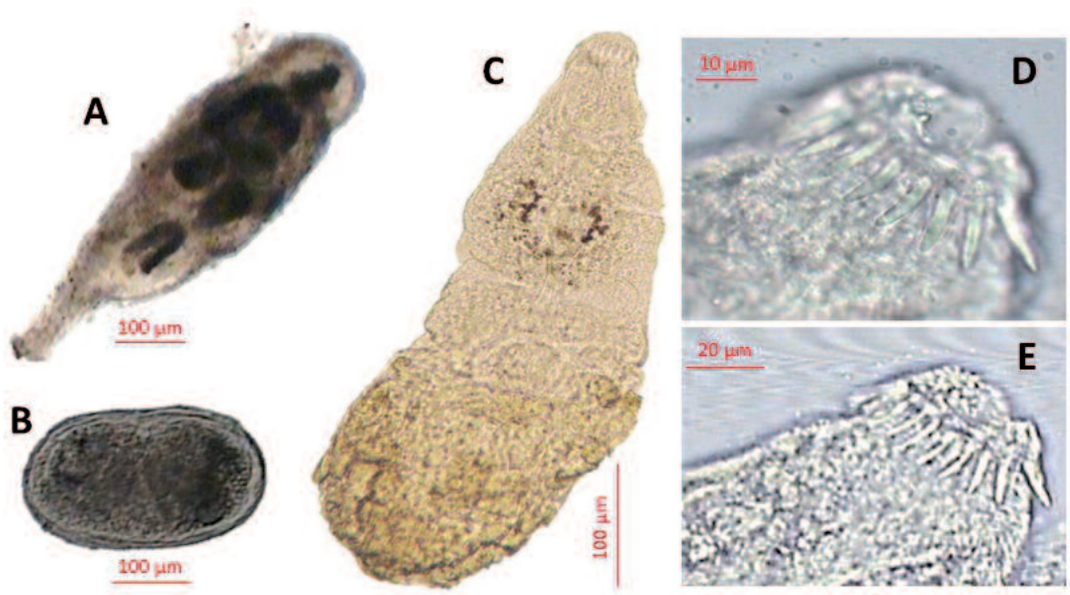

Fig. 1. *Ascocotyle* (*Phagicola*) *longa* from the stomach and intestine of *Chelon auratus* and *Chelon saliens* captured in the Caspian Sea, Iran. A. Multiple cysts; B. Single cyst; C. Excysted metacercaria; D and E. One row of circumoral spines.

Table 2. Mean intensity ± SD, mean abundance ± SD and prevalence (%) of *Ascocotyle* (*Phagicola*) *longa* in *Chelon auratus* and *Chelon saliens* fish

Locality	Fish species	Date of collections	No. of fish	Weight of fish (g)	Total length of fish (cm)	Mean intensity ±SD	Mean abundance ±SD	Prevalence (%)
Anzali	*C. saliens*	2017	24	205.66	31.5	11.5±9.08	3.83±7.46	33
		2018	24	227.75	30.9	11±14.03	5.5 ±11.21	50
	C. auratus	2017	20	245.10	33.06	41	8.2±16.82	20
		2018	16	217.06	30.9	194±225.82	121.25±200.02	63
Kiashahr	*C. saliens*	2017	26	157.18	29.06	1.5±0.57	0.23±0.58	11
		2018	20	282.65	33.36	0	0	0
	C. auratus	2017	16	185.89	29.47	1.71±0.75	0.75±1	44
		2018	12	327.99	35.5	0	0	0

microscopic studies, the trematodes were separated from 10% formalin and placed in physiological saline for 24 hours. In the next step, parasites were washed and dehydrated through graded ethanol series (from 50% to 100% ethanol) and acetone and then mounted on the metallic stub. The prepared samples were coated with gold in a sputtering chamber (SCDOOS, BalTec, Switzerland), and observed by scanning electron microscope (JSM-6380 (JEOL, Tokyo, Japan)), accelerating voltage was used 15 kV.

At last, the quantitative information included prevalence, mean intensity and mean abundance of metacercariae were calculated according to Bush et al. [18] in the Quantitative Parasitology; QP 3.0 Program, available free online [19]. Differences in the mean intensity between sampling years, fish species and locations of mullets were tested by the Kruskal-Wallis test (nonparametric analysis of variance, ANOVA). Statistical analyses were performed using SPSS Version 25.0 statistic software. The significance level was considered 5%.

Results

According to the already mentioned assessment of 158 mullet fish (*Chelon auratus* and *Chelon saliens*), we found that the stomach and intestine of mullets were infected with *Ascocotyle (Phagicola) longa* metacercaria, which is reported in the Caspian Sea for the first time. The morphological features of the metacercaria (encysted and excysted) and biometrical measurements were described in Fig. 1 and Table 1. The most of the metacercaria have multiple or single types of cysts (Fig. 1A,B) surrounded by a membrane. Cyst oval, 311 (253–372) long and 218 (214–261) wide. Whole body spinous and pyriform, 504 (483–653) long and 212 (201–318) wide and its oral sucker, 40 (24–55) long and 27 (21–39) wide is large and a single row of spine 16 (15–18) is around it (Figs. 1D,E and 2), with a lot of eyespots around the pharynx bowed to the posterior, the prepharynx is long, pharynx is muscular and oesophagus quite short, spherical ventral sucker with 34 (24-46) long and 25 (20–34) wide is developed and located in half-posterior of body (Fig.1C). The morphological characteristics of the parasite reveal that the mullet fish are infected with the *Ascocotyle (Phagicola) longa.*

Overall the prevalence, mean abundance and mean intensity of infection were 28.5%, 14.8±71.9

Table 3. Comparison of mean intensity, mean abundance and prevalence (%) of *Ascocotyle* (*Phagicola*) *longa* metacercariae infections in mullet species, locations and sampling years

	Mullet species		Sampling years		Locations	
	C. auratus	*C. saliens*	2017	2018	Anzali	Kiashahr
N	64	94	86	72	84	74
Mean intensity ±SD	100.7±177.3	9.5±11.5	11.9±15.2	94.1±175.09	68.4±144.3	1.6±0.67
Mean abundance ±SD	33.06±110.70	2.4±7.1	3.1±9.4	28.7±104.76	27.7±97.1	0.24±0.63
Prevalence	32.81	25.5	26.7	30.6	40.5	14.9

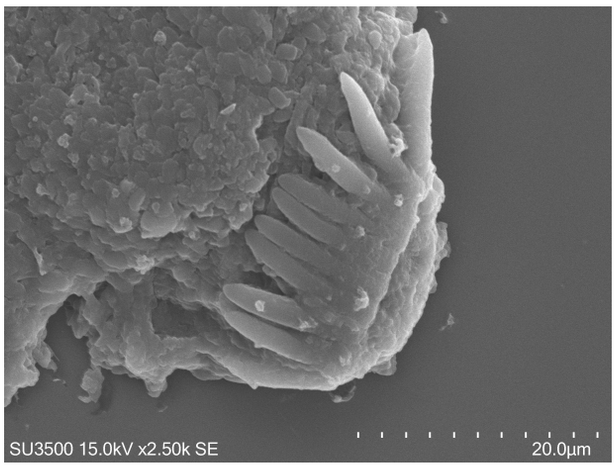

Fig. 2. SEM micrograph of *Ascocotyle* (*Phagicola*) *longa* oral sucker with a single row of spine

and 52.1±128.3 helminths per host, respectively. Table2 indicated the quantitative value of *Ascocotyle (Phagicola) longa* infected mullets in two species, locations and sampling years. Table 3 also compared their infection levels. A Kruskal-Wallis test showed that the mean intensity of infection in Anzali and Kiashahr locations (X^2 = 13.35, df = 1, p = 0.00 < 0.05) were significant and infection rate in Anzali location was higher than the Kiashahr area**.** It was also revealed that the mean intensity between *Chelon auratus* and *Chelon saliens* (X^2 = 5.49, df = 1, p = 0.019 < 0.05) is significantly different. Parasitic infection levels in different sampling years did not show significant differences in intensity of infection (X^2 = 1.95, df = 1, p = 0.16 > 0.05).

Discussion

In this study, the subject of infection of the mullets (*Chelon auratus* and *Chelon saliens*) of the Caspian Sea with *Ascocotyle* (*Phagicola*) *longa* helminth as the fish-borne zoonotic parasite is covered. The second intermediate host of *A.* (*Phagicola*) *longa* is fish, especially the Mugilidae. The consumption of raw fish and infected products by humans can make the consumers involved and result in serious health issues. The mullet fish have metacercariae and no adult parasites were observed. Metacercariae were isolated from the stomach and intestine in the form of the multiple or single cysts. Studies on heterophyid parasites in Iran can be referred to as Massoud et al. [20], as well as Shoaibi Omrani et al. [21] and Farahnak et al. [22]. *Ascocotyle coleostoma* was reported from mullet fish (*Mugil capito* and *Mugil saliens*), in the Caspian Sea by Mikailov [23]. Interestingly, there is a Similar report of *Ascocotyle coleostoma* parasite from the two regions of Turkmenistan and Azerbaijan[24,25]. Recently, Mamedova and Veliyeva [26] have reported *Ascocotyle coleostoma* from shemaya (*Alburnus chalcoides*) gill and heart in the coastal waters of the Absheron Peninsula on the border of the Middle and South Caspian Sea in Azerbaijan.

All specifications for metacercaria in this research are Similar to those given for *A.* (*Phagicola*) *longa* by Simões et al. [9], Martorelli et al. [17] and Santos et al. [7], reported in the same host.

In spite of the wide geographical distribution of *A.* (*Phagicola*) *longa* around the world, and in particular to Mugil genera [3], so far, there are not so many reports of the infection of gold grey mullet. The first reportage of the golden grey mullet infection with *Ascocotyle* (*Phagicola*) *longa* was presented by Özer and Kırca [12] from Kızılırmak Delta wetlands in Turkey, and the prevalence and mean intensity of infection was 6.52% and 6±3.05, which is less than the prevalence (28.5%), mean intensity (52.1±128.3) and mean abundance (14.8±71.9) of infection in this study. Another study by Özer and Kırca [11] is carried out in the same region on the grey mullet (*Mugil cephalus*) that the prevalence (19.68%), mean intensity (7.48±2) and mean abundance (1.47±0.43) were also observed. Besides, Dmitrieva and Gaevskaya [27], reported *Ascocotyle* (*Phagicola*) *longa* from *Mugil cephalus* in Azov and the Black Sea. Santos et al. [7] observed the stomach wall of *Mugil liza* infected with the *Ascocotyle* (*Phagicola*) *longa* and had a relatively high prevalence (77%).

Genus *Phagicola* had not been reported in Iran. These two fish species *C. saliens* and *C. auratus* are very important in the fish market. *A.* (*P.*) *longa* will be alive at a lower temperature for 3 days, if fish consumed freshly, this parasite so causing zoonotic infection in humans [7]. Therefore because of the high prevalence and intensity of this parasite in mullet fish, the risk of human infection is high and it needs more attention to prevent from spreading. Thus, we emphasize adopting local plans of preventing the parasites from transferring to a cage culture and the crucial matter of health management.

Acknowledgements

This study was funded by the Iran National Science Foundation, Science deputy of the

presidency [project number 96015681]. The authors are grateful for this valuable support.

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Received 20 July 2019
Accepted 02 September 2019