

HISTOPATHOLOGICAL ASPECTS CAUSED BY NEMATOD PARASITE *Eustrongylides excisus* OBSERVED IN SOME FISH SPECIES OF EĞİRDİR LAKE, TURKEY

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Abstract

The present study was carried out between January and October 2018, in order to determine the infection of *Eustrongylides excisus* Jägerskiöld, 1909 in some fish species inhabiting Eğirdir Lake - the second largest freshwater lake of Turkey. Parasite infection was detected in host species belonging to Konya tooth-carp (*Aphanius iconii* Akşiraz, 1948), Big-scale sand smelt (*Atherina boyeri* Risso, 1810), Caucasian dwarf goby (*Knipowitschia caucasica* Berg, 1916) and pike-perch (*Sander lucioperca* Linnaeus, 1758). The highest prevalence of infection (21.4%) was determined in *S. lucioperca*. The mean intensity ranged from 1.25 nematode/fish in *K. caucasica* to 2.3 nematodes/fish, in *S. lucioperca*. The nematode *E. excisus* was recorded for the first time on Caucasian dwarf goby, a non-native fish species in Eğirdir Lake. In addition, this research provides useful data regarding histopathology effects of *E. excisus* in all studied teleost species. Hyperaemia, edema, microhaemorrhages, inflammatory reaction and necrosis were observed around the encysted parasites.

Key words: alien, endemic, *Eustrongylides excisus*, histopathology, Turkey.

INTRODUCTION

The worldwide parasite *Eustrongylides excisus* Jägerskiöld, 1909 is considered a freshwater zoonotic nematode potentially dangerous for human health (Branciari et al., 2016; Goncharov et al., 2018). In the life cycle of this worm, fish are intermediate hosts for the larvae which develop in muscles, within the body cavity, liver or other visceral organs, while wading birds species such as herons, egrets or cormorants are the definitive hosts (Bjelić-Čabrilo et al., 2013).

The pike-perch or other piscivorous fish species become infected when consuming the already parasitized prey and could play the role of paratenic host to the nematodes, when ingested by birds (Soylu, 2013; Dezfuli et al., 2015). For humans, accidentally eating of undercooked or raw fish infested with larvae means that *E. excisus* become pathogenic, causing gastritis and verminous peritonitis

(Goga and Codreanu-Bălcescu, 2013; Ljubojevic et al., 2015; Branciari et al., 2016). Novakov et al. (2013) indicated the need for adequate preparation of fish meat as key factor in combating eustrongylidiosis.

Fish infections with *E. excisus* in Turkey were reported from various host species: *Neogobius fluviatilis* from lakes Manyas (Ozturk et al., 2001) and Uluabat (Ozturk et al., 2002); *Silurus glanis* from Durusu (Terkos) Lake (Soylu, 2005); *Abramis brama* from Durusu (Terkos) Lake (Karatoy and Soyly, 2006); *Aphanius mento* from Kırkgöz Stream (Aydoğdu et al., 2011); *Perca fluviatilis* and *Sander lucioperca* in lake Sığırcı (Çolak, 2013); *Atherina boyeri* from Iznik Lake (Özesen Çolak, 2013); *Carassius gibelio*, *Cyprinus carpio*, *Sander lucioperca* and *Atherina boyeri* from lake Eğirdir in Turkey (Akcimen et al., 2014); *Sander lucioperca* in Lake Eğirdir (Metin et al., 2014); *Cyprinus carpio* from Marmara Lake (Demir, 2014) and

Carassius gibelio from Marmara Lake (Demir and Karakişi, 2016); *Perca fluviatilis* and *Lepomis gibbosus* from Gala Lake (Soylu, 2014); *Oncorhynchus mykiss* (Akcimen et al., 2016); *Silurus glanis*, *Perca fluviatilis*, *Leuciscus cephalus*, *Rutilus rutilus*, *Vimba vimba*, *Scardinius erythrophthalmus* from Büyükçekmece Dam Lake (Yardimci et al., 2018); *Capoeta baliki* from Seydi River (Mnisi, 2017); *Vimba vimba*, *Scardinius erythrophthalmus* from Balikhane Creek (Yazıcı, 2013) and *Proterorhinus marmoratus* from Kurtköy Creek (Yazıcı, 2013); *Alburnus chalcoides* and *Lepomis gibbosus* from Lake Mert (Kırcalar, 2018); *Aphanius iconii* from Eğirdir Lake, *A. saldae* from Salda Lake, *A. sureyanus* from Burdur Lake, *A. transgadiens* from Acıgöl Lake (Innal et al., 2019).

E. excisus was reported so far in freshwater Eğirdir Lake in *Aphanius iconii*, *Cyprinus carpio*, *Atherina boyeri*, *Carassius gibelio*, *Sander lucioperca* (Akcimen et al., 2014; Metin et al., 2014; Innal et al., 2019) fish species.

Belonging to Aphaniidae monophyletic family group of Cyprinodontiformes, Konya tooth-carp *Aphanius iconii* Akşiraz, 1948 is a widely fish species distributed in freshwater Lake Eğirdir basin from Isparta province, Turkey (Freyhof et al., 2017; Esmaeili et al., 2018). This species is considered to be endemic for Eğirdir and Kovada Lakes (Yoğurtçuoğlu and Ekmekçi, 2017). The parasitofauna of this fish species has remained unknown until recently. In a previous study, Innal et al. (2019) recorded the presence of monogenean *Salsuginus* sp., crustaceans (*Argulus foliaceus*, *Lernaea cyprinacea*) and nematode *E. excisus* on *A. iconii* host species from Eğirdir Lake.

Big-scale sand smelt *Atherina boyeri* Risso, 1810 (Atheriniformes, Atherinidae) is a euryhaline species mainly encountered in coastal and estuarine waters, considered an exotic fish for Turkish inland waters (Küçük et al., 2012). *A. boyeri* was introduced by fishermen in Eğirdir Lake for its commercial value. Growth and reproduction of this important fish in Eğirdir Lake were clarified before by Yağcı et al. (2017), the literature review indicated little research conducted on sand smelt's parasites in this study area

(Akcimen et al., 2014). Caucasian dwarf goby *Knipowitschia caucasica* Berg, 1916 (Perciformes, Gobiidae) is considered to be an alien species for the Lake Eğirdir. According to Güçlü and Erdoğan (2017), *A. boyeri* and *K. caucasica* are nowadays among the major fish species of this lentic ecosystem. Another non indigenous species, the pike-perch *Sander lucioperca* Linnaeus, 1758 (Perciformes, Percidae) drastically changed the native fish fauna of Lake Eğirdir after its intentional introduction in 1955 (Güçlü and Erdoğan 2017). Diler and Yıldırım (2003) have mentioned the infestation of *S. lucioperca* and *K. caucasica* from Lake Eğirdir by trematode *Bucephalus polymorphus*. Metin et al. (2014) reported larval forms of *E. excisus* in abdominal cavity, peritoneum, muscle, adipose tissue, liver, spleen, swimbladder and stomach of *S. lucioperca* from Lake Eğirdir, and also highlighted the importance of public awareness concerning such fish consumption.

Although the presence of *E. excisus* has been described in several fish hosts worldwide, studies based on pathological effects of this nematode species remain scarce. To date, the only available data about the pathological findings of *E. excisus* larvae infection in fish from Turkey were recorded in native species of genus *Aphanius* (Innal et al., 2019). Therefore, the aim of this paper was to investigate the pathological effects and occurrence of nematode *E. excisus* parasitizing some native and introduced fish species of Eğirdir Lake.

MATERIALS AND METHODS

This study was conducted between January 2018 and October 2018 in fishes inhabiting Eğirdir Lake (Figure 1). Located at 38°15' latitude N and 30°52' longitude E in the Lake District from southwest Turkey, Lake Eğirdir is the second largest freshwater lake of Turkey (Güçlü 2012).

Three stations (Yeşilada, Bedre and Boyalı) were selected and samplings were carried out by beach seine nets for *Aphanius iconii*, *Atherina boyeri* and *Knipowitschia caucasica*. *Sander lucioperca* specimens were purchased from a commercial fisherman.

Fish individuals were placed in a well aerated 20-litre aquarium filled with stream water. Fish

samples were maintained in the aquarium for 2-3 hours and subsequently anaesthetized by MS-222.

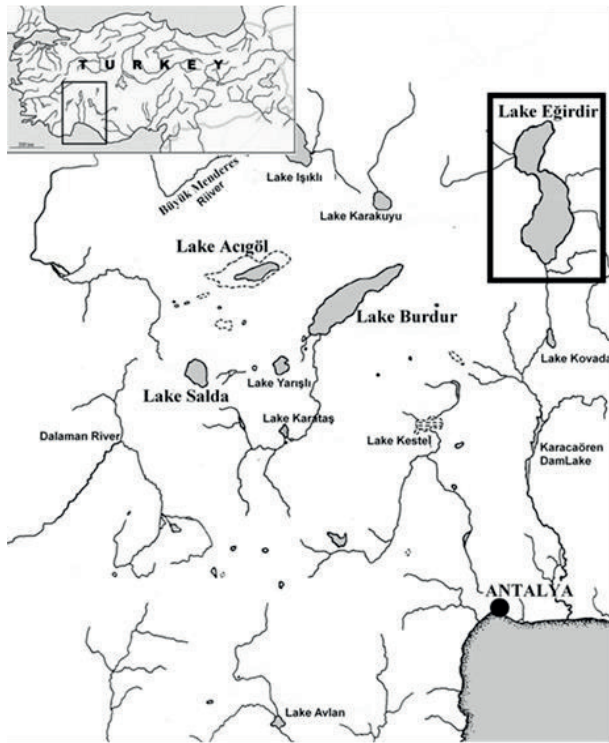


Figure 1. Study area for fish sampling (Eğirdir Lake)

Fish samples were measured and weighed to the nearest 1.0 mm and 0.1 g, respectively. During the dissection, internal organs (gastrointestinal tract, liver, kidney, heart, swim bladder and gallbladder), and body surfaces were examined separately under a dissecting microscope.

Fixation, staining and preparation process of the determined parasites was done according to Pritchard and Kruse (1982). The parasites were identified using selected identification keys (Markevich, 1951; Bykhovskaya-Pavlovskaya, et al., 1962; Burton, 1984; Bauer, 1987; Moravec, 1994).

Prevalence and mean intensity were calculated for each parasite species as defined by Bush et al. (1997). Some fish were selected for histopathological examination. Because the fish and organs were small, the whole body was transversally cut into six pieces from head to tail (Koca et al., 2019). Carefully slicing performed near the parasite attached areas. After slicing all parts of the fish were fixed in buffered 4% formaldehyde. Tissue samples were processed by automatic equipment (Leica ASP300S, Wetzlar, Germany) and embedded in

paraffin. Sections (5 μ m) were cut by a Leica RM2155 (Wetzlar, Germany) rotary microtome and mounted on glass slides before staining with Hematoxylin and Eosin (HE).

Stained sections were examined under light microscopy (Olympus CX41, Tokyo, Japan). Morphometric evaluation and microphotography was performed using the Database Manual cellSens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

RESULTS AND DISCUSSIONS

A total of 89 *Aphanius iconii*, 411 *Atherina boyeri*, 85 *Knipowitschia caucasica* and 28 *Sander lucioperca* individuals were inspected for the presence of parasites. In all examined fish species, only nematode *Eustrongylides excisus* was identified (Table 1). Overall, 51 specimens of *E. excisus* larvae were collected from 34 of 613 fish individuals. The highest prevalence of infection (21.4%) was determined in *S. lucioperca*. The prevalence of infection in *A. iconii*, *A. boyeri* and *K. caucasica* (Figure 2) populations in Eğirdir Lake was nearly similar (3.4%, 5.1% and 4.7%, respectively). The mean intensity ranged from 1.25 nematode/fish in Caucasian dwarf goby to 2.3 nematodes/fish, in pike-perch (Table 2).

At the microscopical examination, larvae of *E. excisus* were in generally localized in the abdominal cavity but numerous parasites were also detected in the muscles of analyzed fish species. Most of the nematodes were found near the ovaries and hepatopancreas. In some cases, intestinal sections revealed the presence of numerous parasites. The parasites were covered by a thin fibrous tissue and inflammatory cell infiltrations were commonly observed around the parasites. In some cases, hyperemia, microhemorrhage and edema were present. Marked necrosis was observed in severe cases and more than one parasite localized in same area (Figure 3).

Of the host species of interest for this study, *E. excisus* have been founded so far in Eğirdir Lake on alien fish species *Atherina boyeri* and *Sander lucioperca* (Akcimen et al., 2014) and on native *Aphanius iconii* (Innal et al., 2019). Metin et al. (2014) detected the occurrence of the nematode in pike-perch populations of this

Turkish inland water, however histopathology aspects were missing.

As novelty, the present work provides microphotographs of *Eustrongylides excisus* encysted in four fish species of Eğirdir Lake. In addition, this study reports for the first time the presence of *E. excisus* in Eğirdir Lake on alien host *K. caucasica* and for the second time on endemic fish species *Aphanius iconii*.

In a previous study (Innal et al., 2019) concerning the parasitofauna of fish caught in

Eğirdir Lake, *A. iconii* showed 2.8% prevalence and 1.25 mean intensity of infection with *E. excisus* larvae. Özesen Çolak (2013) studied the metazoan parasites of 271 specimens of *A. boyeri* in Iznik Lake between June 2008 and May 2009 and for *E. excisus* infection recorded the following data: overall prevalence and mean intensity 6.64% and 1.2, respectively; no nematode larvae found from July to October; zero prevalence in fish from 3.0-3.9 to 7.0-7.9 cm.

Table 1. Host species and their status in ichthyofauna of Eğirdir Lake

Species	Family	Status	N	L, cm (min.-max.)	W, g (min.-max.)
<i>Aphanius iconii</i>	Aphaniidae	Endemic	89	2.3-4.3	0.18-1.5
<i>Atherina boyeri</i>	Atherinidae	Introduced	411	4-6.4	0.15-1.8
<i>Knipowitschia caucasica</i>	Gobiidae	Introduced	85	2.2-3.5	0.14-0.48
<i>Sander lucioperca</i>	Percidae	Introduced	28	23.6-42.5	112-710

Table 2. Prevalence and intensity of *E. excisus* infection in host fish species from Eğirdir Lake (2018)
(N = total number of hosts examined; N' = number of infected fishes; NP = number of collected parasites;
P = prevalence; Int = mean intensity of infection)

Species	N	N'	P (%)	NP	Int.
<i>Aphanius iconii</i>	89	3	3.4	5	1.7
<i>Atherina boyeri</i>	411	21	5.1	27	1.3
<i>Knipowitschia caucasica</i>	85	4	4.7	5	1.3
<i>Sander lucioperca</i>	28	6	21.4	14	2.3



Figure 2. *Eustrongylides excisus* in *K. caucasica* host

According to Diler and Yıldırım (2003), the pike-perch is the main catch of the fisherman in Eğirdir Lake. This fact is important, since fish-borne nematodes belonging to genus *Eustrongylides* could represent a threat to carnivorous organisms that ingest the parasites (Eiras et al., 2016; Shaw, 2018).

It was postulated before that high prevalence of infection of *S. lucioperca* in Eğirdir Lake has a negative impact on the marketability of this fish species (Metin et al., 2014). There is little knowledge about the pathological reaction against to *Eustrongylides excisus* in fish. In this study were noticed hyperaemia, edema, slight hemorrhage, inflammatory reaction and in some cases necrosis in the host tissues caused

by the parasite. These findings were in agreement with our previous study (Innal et al., 2019). Similar inflammatory response of *A. boyeri* was recorded by Branciarri et al. (2016) on fish infected with *Eustrongylides* spp. in Trasimeno Lake, Italy. Metin et al. (2014) noticed abdominal swelling, hemorrhage in jaw, fins, gills, liver, intestine, spleen and muscles of *S. lucioperca* in Eğirdir Lake. Also, hemorrhages and inflammatory reactions due to *E. excisus* infection were registered in *A. iconii* of Eğirdir Lake (Innal et al., 2019). In this study it was found that severe lesions appeared when more than one parasite infected the same area.

So far, the only known parasite species of ichthyofauna inhabiting the Eğirdir Lake are: the cestode *Ligula intestinalis* Linnaeus, 1758; the trematode *Bucephalus polymorphus* Baer, 1827; the monogenean *Salsuginus* sp.; the nematode *Eustrongylides excisus* Jägerskiöld, 1909) and crustaceans *Argulus foliaceus* L., 1758 and *Lernaea cyprinacea* L., 1758 (Güralp, 1968; Diler and Yıldırım, 2003; Innal et al., 2007; Metin et al., 2014; Innal et al., 2019).

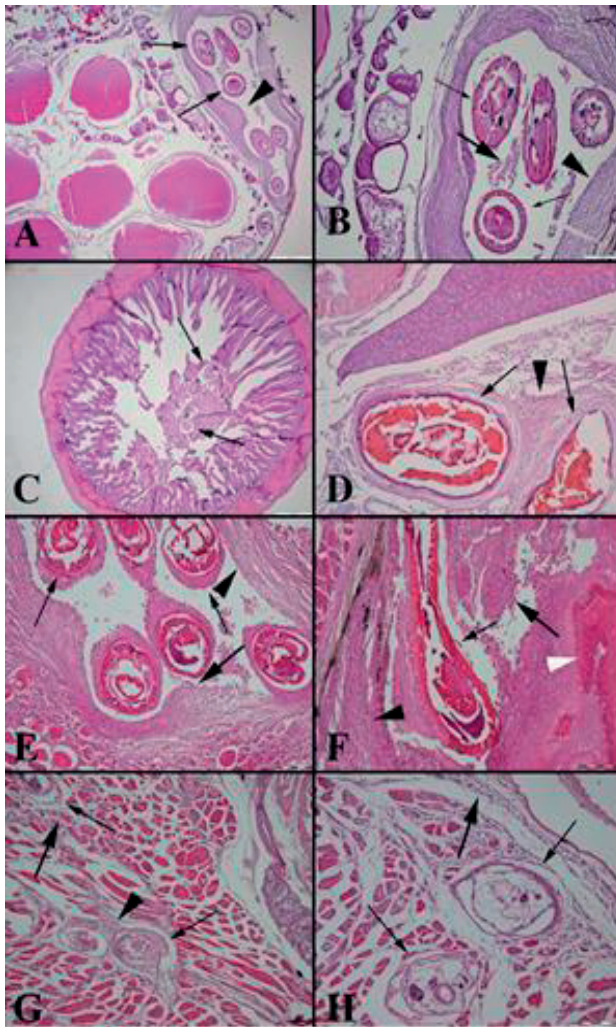


Figure 3. Main sites of location for nematode *Eustrongylides excisus* within its hosts (A, B - *Aphanius iconii*; C, D - *Sander lucioperca*; E, F - *Atherina boyeri*; G, H - *Knipowitschia caucasica*) from Eğirdir Lake: (A) Parasite (arrows) localized in abdominal cavity. The nematodes are separated by a fibrous capsule from the fish visceral organs, near the ovarian follicles, Bar = 500 µm; (B) Inflammatory reaction (thick arrow) around the parasites (thin arrows) covered by a fibrous capsule (arrow head), HE, Bar = 200 µm; (C) Numerous parasite sections (arrows) in an intestinal section of the host, Bar = 500µm; (D) Two parasite sections near the pancreatic tissue. Fibrous tissue (arrow heads) around the parasite (arrows) and inflammatory cell reaction is visible, Bar = 200 µm; (E) Numerous parasites (thin arrows) localized in muscles and covered by fibrous capsule (arrow head) and inflammatory reaction (thick arrow) around the nematodes, Bar = 200 µm; (F) Another parasite individual (thin arrow) localized in muscle caused marked inflammatory reaction (thick arrow) and necrosis (white arrow head) covered by fibrous capsule (black arrow head), Bar = 200 µm; (G) Nematodes (thin arrows) localized in muscle, covered by fibrous tissue (arrow head) and slight inflammatory reaction (thick arrow), Bar=200 µm; (H) Parasites (thin arrows) localized in muscles caused moderate inflammatory reaction (thick arrow), Bar = 100 µm, HE

CONCLUSIONS

The novelty of the present work lies in providing for the first time microphotographs of nematode parasite *E. excisus* encysted in four fish species of Eğirdir Lake: one endemic (*A. iconii*) and three introduced (*A. boyeri*, *K. caucasica*, *S. lucioperca*).

Moreover, for freshwater Eğirdir Lake the first record of *E. excisus* on alien host *K. caucasica* and the second record on endemic fish species *A. iconii* were highlighted in this study.

Further studies are encouraged in searching of new parasite species in endemic and introduced fish of the second largest freshwater lake in Turkey.

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