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Original research

Occurrence and infection dynamics of *Salsuginus* sp. (Monogenea, Ancyrocephalidae) and *Eustrongylides excisus* (Nematoda, Dioctophymatidae) in four endemic *Aphanius* (Cyprinodontidae) species

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Abstract: This paper aims to determine the occurrence of *Salsuginus* sp. and *Eustrongylides excisus* in four endemic fish species for Turkish fauna. A total of 326 host individuals were collected from the lentic ecosystems Acıgöl Lake, Salda Lake, Burdur Lake and Eğirdir Lake. For both parasite taxa, prevalence and mean intensity were estimated in each host species. This study reports for the first time the presence of *E. excisus* in four *Aphanius* species.Also, to the best of our knowledge, *Aphanius transgrediens, A. saldae, A. iconii* and *A. sureyanus* represent new host records for the presence of the monogenean *Salsuginus* sp. In addition, this study showed that *E. excisus* can cause pathological findings in host such as hyperaemia, haemorrhages and inflammatory reactions.

Keywords: pathology, histology, endemic, threatened, biodiversity

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Introduction

In recent years, the distribution and systematics of *Aphanius* have undergone numerous taxonomic changes and intensive investigations in Turkey. According to Çiçek et al. (2015) there are 14 representatives of the genus *Aphanius* in Turkey. Except *A. fasciatus* and *A. mento* all species of *Aphanius* genus are endemic to Anatolia. *Aphanius iconii, A. saldae*, *A. sureyanus* and *A. transgrediens* are found in highly localized populations. They are affected by anthropogenic pressures of habitat modifications and biological invasions (Innal D., pers. Observation)

The available information on *Aphanius* genus in the specialist literature mainly focuses on taxonomy and distribution (Wildekamp et al., 1999; Pfleiderer et al., 2014; Yoğurtçuoğlu and Ekmekçi, 2017; Freyhof et al., 2017), its growth properties (Güçlü et al., 2007; Karslı and Aral, 2010; Güçlü 2012; Yoğurtçuoğlu and Ekmekçi, 2013), genetic structure (Cavraro et al., 2017) and aspects of its phylogenetic development and biogeography (Hrbek et al., 2002; Bardakçı et al., 2004). There have been only few studies on the parasites of *Aphanius* fish species in Turkey (Özer, 2007; Öztürk and Özer, 2007; Öztürk and Özer 2008; Aydoğdu et al., 2011; Öztürk and Özer, 2014; Smales et al., 2015). As Öztürk and Özer (2014)

highlighted, data on the occurrence of *Salsuginus* on *Aphanius* species are scarce. Of *Aphanius* fish genus, *Salsuginus* sp. was previously recorded infesting only *A. danfordii* species from Sarıkum Lagoon Lake (Öztürk and Özer, 2008). On the other side, *A. mento* from Kırkgöz Stream (Antalya) is only species of *Aphanius* genus that was cited so far to host larvae of *Eustrongylides excisus* in the abdominal cavity (Aydoğdu et al., 2011).

The survey of parasites in threatened fishes is required for the management and conservation of fish populations in natural water bodies. In the official status of Red List of Threatened Species, *A. sureyanus* is listed as Endangered (Freyhof, 2014a), whilst *A. transgrediens* is Critically Endangered species (Freyhof, 2014b). Threats status of *A. iconii* and *A. saldae* has not been determined. To the best of the authors' knowledge, there are no parasitological data for *A. iconii, A. saldae*, *A. sureyanus* and *A. transgrediens*.

It is documented that findings of *Eustrongylides* sp. is important in fish, since these aquatic organisms act as intermediate hosts in the lifecycle of the nematode (Ljubojevic et al., 2015). Zoonotic potential of *Eustrongylides* sp. has been reported by various authors (Abe, 2011; Branciari et al., 2016; Melo et al., 2016).

Moreover, infestation of nematode in the female fish reduces the fecundity which may further conduct to decline in the host fish population (Kaur et al., 2013). Other fish host species cited so far in Turkey for the nematode *Eustrongylides excisus* included freshwater bream *Abramis brama,* wels catfish *Silurus glanis,* monkey goby *Neogobius fluviatilis,* pearl-spotted killifish *Aphanius mento*, European perch *Perca fluviatilis,* pike-perch *Sander lucioperca,* common carp *Cyprinus carpio* and big-scale sand smelt *Atherina boyeri* (Öztürk et al., 2001; Soylu, 2005; Karatoy and Soylu, 2006; Aydoğdu et al., 2011; Çolak, 2013a; Akcimen et al., 2014; Metin et al., 2014).

Although it is known that parasites can cause pathological effects in their hosts, there is no knowledge about occurred lesions caused by *E. excisus* in *Aphanius*. This research aimed to investigate the occurrence of parasites of *A. iconii, A. saldae*, *A. sureyanus* and *A. transgrediens* in Turkey. The study could serve as a database for future helminthological and ecological work. In addition, this study is a preliminary pathological study about *E. excisus* in *Aphanius.*

Materials and Methods

A total of 326 fish individuals belonging to Cyprinodontidae family were caught with shore seine netting during Spring of 2014 and Winter of 2015, as follows: *A. transgrediens* (n= 71, Lake Acıgöl); *A. saldae* (n= 65, Lake Salda); *A. sureyanus* (n= 47, Lake Burdur) and *A. iconii* (n= 143, Lake Eğirdir). Locations of the Lake Acıgöl, Lake Salda, Lake Burdur and Lake Egirdir are shown in Figure 1.

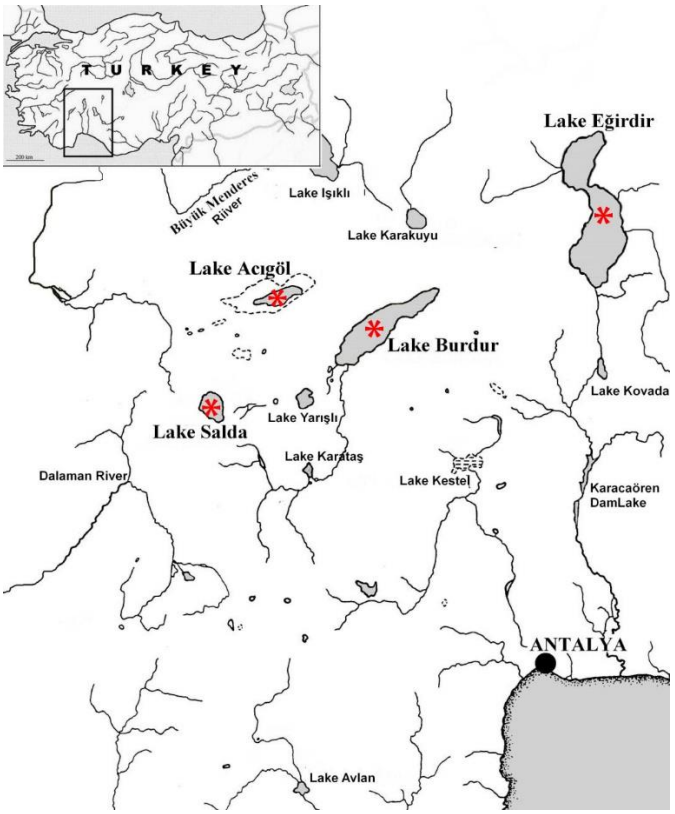

Figure 1. Map indicating the locations of sampling sites

Specimens were placed in a well aerated 20-litre aquarium filled with stream water. The fish were maintained in the aquarium for 2-3 hours and subsequently anaesthetized by MS-222. The total length was measured and sex determined at necropsy by macroscopic investigation. During the dissection, internal organs (gastrointestinal tract, liver, kidney, heart, swim bladder and gallbladder), gill filaments, eyes, fins, 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). All parasites were identified using selected identification keys (Markevic (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).

The fishes totally fixed in a buffered 4% formaldehyde solution for histopathological examination and then divided in to 5 parts throughout the body. Specimens were processed by an automatic tissue processor equipment (Leica ASP300S, Wetzlar, Germany) and embedded in paraffin. Sections (5µm) were cut by a Leica RM2155 (Wetzlar, Germany) rotary microtome and mounted on glass slides before staining with Hematoxylin and Eosin (H&E). Stained sections were examined under light microscopy (Olympus CX41, Tokyo, Japan). Morphometric evaluation and microphotography was performed using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

A Kruskal-Wallis test was applied to find significant differences in the mean intensity of the parasite species for host fish sex and seasons. The differences in parameters were considered significant at $p < 0.05$.

Results

Prevalence of each parasite infections is shown in Table 1. Two parasite species were identified from *Aphanius iconii*, *A. saldae*, *A. sureyanus* and *A. transgrediens*: *Salsuginus* sp. (Monogenea) in the gills and *Eustrongylides excisus* (Nematoda) in the intestine. Besides the presence of monogenean *Salsuginus* sp. and nematode *Eustrongylides excisus*, *A. iconii* was detected as host also for crustaceans *Argulus foliaceus* and *Lernaea cyprinacea*.

Table 1. Prevalence of parasite infections in *Aphanius* hosts

Host Species	N	TL (min-max) (cm)	TW (min-max) (g)	Prevalence (%)				
				Argulus foliaceus	*Lernaea cyprinacea*	*Salsuginus* sp.	*Eustrongylides excisus*	Total infection
A. transgrediens	71	2.0-6.0	0.091-2.331	-	-	5.63	15.49	21.13
A. saldae	65	3.8-5.2	0.41-2.09	-	-	3.08	7.69	10.77
A. sureyanus	47	1.4-4.5	0.029-0.947	-	-	4.26	10.64	14.89
A. iconii	143	2.1-4.5	0.16-1.56	2.1	2.1	2.1	2.8	9.09

The prevalence of parasites according to the host sexes are presented in Figure 2. Although fish sample of *A. iconii* presented the highest number of individuals (143) from all collected species, the prevalence of overall infection was the lowest (9.09%). The highest prevalence of infection (total, 21.13 %) with both *Salsuginus* and *Eustrongylides* parasites were found in *A. transgrediens* (5.63%, 15.49% respectively). The highest value of mean intensity was 1.25 for *Eustrongylides excisus* infection (*A. iconii*), and 1.5 for *Salsuginus* sp. in *A. transgrediens* and *A. sureyanus* hosts.

In *A. transgrediens* and *A. saldae*, the overall prevalence of the parasite infection was higher in females, while in *A. sureyanus* and *A. iconii*, fish males were more infected. Infection prevalence of female and male individuals are significantly different in *A. sureyanus* ($p < 0.05$).

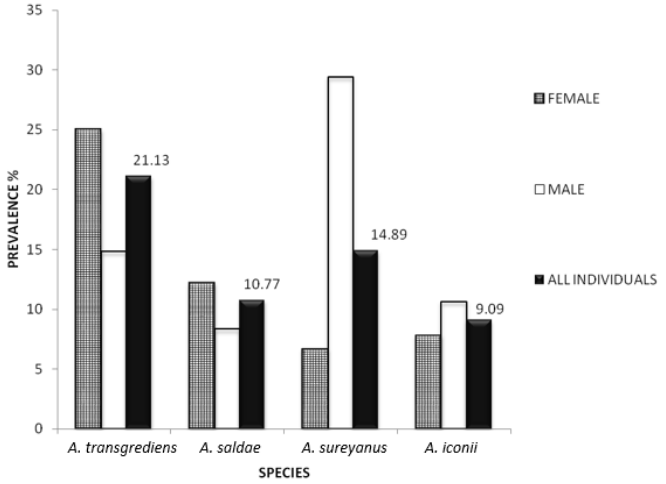

Figure 2. The prevalence and intensity values of parasites according to the host sexes

Seasonal prevalence of infections presented in Table 2. With respect of seasonal fluctuations, the highest incidence of *Salsuginus* sp. occurred in Autumn of 2014 (33.33% and 17.65% in *A. sureyanus* and *A. transgrediens* hosts). The prevalence of *Eustrongylides excisus* was

higher in Spring of 2014 (23.81% in *A. sureyanus*) and Winter of 2015 (50% in *A. saldae*). Number of individuals of *A. saldae*, *A. transgradiens* and *A. sureyanus* were not enough for statistical analysis of seasonal infections. Seasonal prevalence of *Salsuginus* sp. and *Eustrongylides excisus* infection was significantly different in *A. iconii.*

Table 2. The prevalence values of parasites according to seasons

Season	Prevalence (%)			
	A. transgrediens	*A. saldae*	*A. sureyanus*	*A. iconii*
Salsuginus sp.				
Spring 2014	0.00	0.00	0.00	0.00
Summer 2014	4.00	0.00	0.00	4.00
Autumn 2014	17.65	13.33	33.33	15.38
Winter 2015	0.00	0.00	0.00	0.00
Eustrongylides excisus				
Spring 2014	17.65	4.35	23.81	4.76
Summer 2014	16.00	9.00	0.00	0.00
Autumn 2014	11.76	6.67	0.00	23.08
Winter 2015	16.67	50.00	0.00	0.00
Total infection (*Salsuginus* sp. and *Eustrongylides excisus*)				
Spring 2014	17.65	4.35	23.81	4.76
Summer 2014	20.00	8.00	0.00	4.00
Autumn 2014	29.4	20	33.33	38.5
Winter 2015	16.67	50.00	0.00	0.00
Total	21.13	10.77	14.89	4.9

Histopathological examination of the abdominal cavity revealed numerous parasites sections belong to one parasite because of the corrugated localisation. In females most of the parasites localised near the ovaries. Visceral organs of the parasites easily demonstrated at the microscopical examination. In some cases, free parasites were seen in abdominal cavity. The parasites covered by a thin fibrous tissue and inflammatory cell infiltrations commonly observed around the parasites. Histopathological examination revealed that parasites were surrounded by the encapsulations composed of granulomatous reaction and scattered lymphocytes. In some cases, hyperaemia, micro haemorrhages and oedema were present in gonads. Generally, parasite nodule centres full with necrotic debris and inflammatory cells. Mesenterial vessels were severely hyperaemic if free parasites were in the abdominal cavity (Figures 3-5).

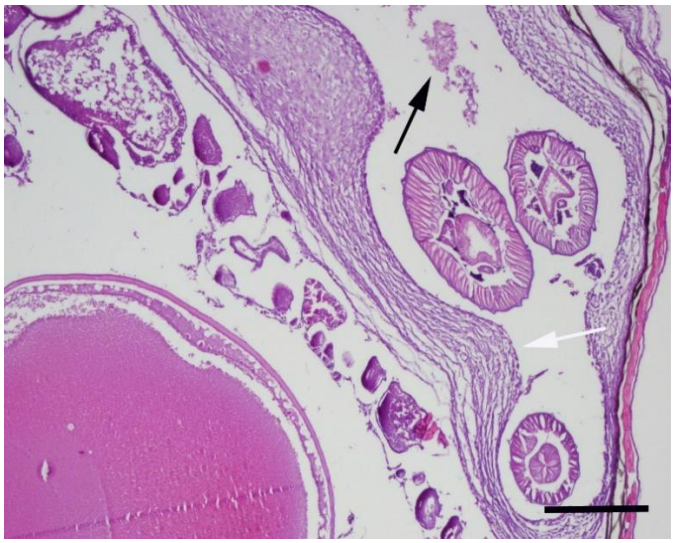

Figure 3. *Eustrongylides excisus* (arrows) localised in an *Aphanius transgradiens*. (A) The parasites separated by a fibrous capsule (white arrow) from the fish ovary of the host and inflammatory reaction (black arrow) around the parasite, HE, Bars= 200 μm.

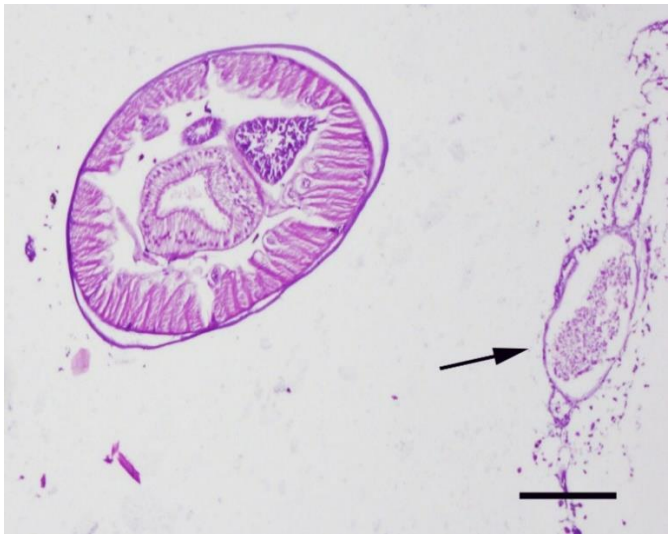

Figure 4. A parasites localised in abdominal cavity of an *Aphanius sureyanus*, marked hyperaemia in the mesenterial vessels (arrow), HE, Bars= 100 μm.

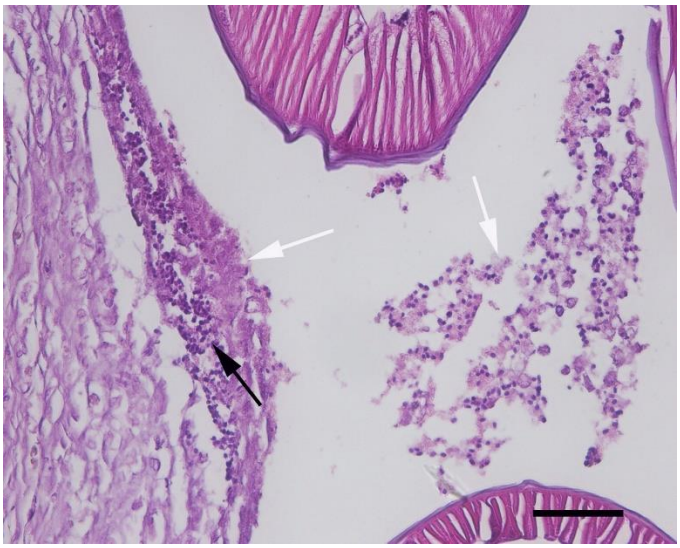

Figure 5. Inflammatory reaction (black arrow) and necrotic debris (white arrows) around the parasites, HE, Bars= 50 μm.

Discussion

According to other Ancyrocephalid species, because of the differences it has in the copulation complex and haptoral sclerites, *Salsuginus* as a new monogenean genus was proposed by Beverley-Burton in 1985. Despite being considered a new genus investigations have shown that *Salsuginus* species are typical gill parasites of Cyprinodontiformes. In Turkey, *Salsuginus* sp. was reported in Sarıkum Lagoon Lake and Lower Kızılırmak Delta populations of *Aphanius danfordii* by Öztürk and Özer (2014). They determined prevalence of *Salsuginus* sp. as 8.8% in 2014. In the present study, the highest average of prevalence was 5.63% and it belongs to *A. transgrediens*. But in both studies the highest prevalence values during the year were noticed in autumn in all *Aphanius* species.

Although more than twenty species of *Eustrongylides* have been described, only three are considered valid at present: *Eustrongylides tubifex*, *E. ignotus* and *E. excisus* (Measures, 1988). *E. ignotus and E. tubifex* are common in North and South America and Europe, while *E. excisus* is common in Asia (Karmanova, 1968). Many piscivorous birds as members of Pelecaniformes, Ciconiiformes and Anseriformes are definitive hosts of this parasite in Europe, Southeast Asia, the Middle East and Australia (Measures, 1988). The prevalence of *E. excisus* was highest (50%) in Winter of 2015 in *A. saldae* from Lake Salda. Kaur et al. (2013) stated that a heavier infection in winter months may be due to decrease in water temperature, related to a reduced immune response of fish and increased vulnerability to nematode infection. Similarly, Çolak (2013b) reported the highest prevalence of this parasite in winter on sand smelt from Lake İznik; the author added that while sand smelt was feed on benthic organisms like *Tubifex* in winter, it preferred pelagic invertebrates in the summer months. Karmanova (1968) reported that development of the egg of *E. excisus* to the first-stage larva occurs in water and takes 21 to 30 days during summer. Then embryonated eggs are swallowed by the intermediate host as freshwater oligochaetes (*Lumbricullus, Tubifex* and *Limnodrilus*). Development of the larvae on this host lasts approximately 70 days and pass to the second intermediate host like benthopelagic fishes. The findings of both authors are complementary quality each other and explain why the parasite prevalence is higher in winter.

The differences in prevalence and intensity of infections can be attributed to biotic and abiotic parameters of lake systems. Öztürk and Özer (2014) draw attention to the fact that monogenean occurrence could be affected by variations in temperature and salinity levels of water body from different geographical areas. With respect of seasonal fluctuations, the highest incidence of *Salsuginus* sp. occurred in Autumn of 2014. Our data are consistent with Öztürk and Özer (2014), which reported that *Salsuginus* sp. preffered the autumn season for infesting *A. danfordii*, in a study regarding the monogenean fauna of fish from Lower Kızılırmak Delta.

There is no knowledge of the pathological findings caused by *Eustrongylides excisus* in *Aphanius* spp. This preliminary study indicated that this parasite caused, hyperaemia, oedema, slight haemorrhage and inflammatory reaction in the host tissues. These findings were in agreement with classical knowledge about parasite pathology (Kaur et al., 2013).

During this parasitological survey, investigated endemic *Aphanius* fish species for Turkish lentic ecosystems lakes Acıgöl, Salda, Burdur and Eğirdir are recorded as new hosts for the monogenean *Salsuginus* sp. and the nematode *Eustrongylides excisus*: *A. transgrediens*, *A. saldae, A. iconii* and *A. sureyanus*. We conclude that further research like this is needed in fisheries management, in order to complete the knowledge regarding the host-parasite lists for endemic and endangered fish species of Turkish lakes.

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