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Research Article

Prevalence and intensity of *Paradiplozoon homoion* (Monogenea: Diplozoidae) from Manyas spirlin, *Alburnoides manyasensis*, an endemic fish of Turkey: new host and geographical record

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Abstract
In this study, the occurrence of a parasitic helminth infecting a Turkish endemic fish, Manyas spirlin, *Alburnoides manyasensis* from the Nilüfer stream, Bursa, was studied from winter 2017 to autumn 2018. A total of 46 *A. manyasensis* were examined for the presence of the helminth. The helminth was identified as *Paradiplozoon homoion* (Monogenea: Diplozoidae) and occurred on the gills of host fish. A total of 115 specimens of *P*. *homoion* infected 32 of 46 fish examined, with prevalence and mean intensity of infection of 69.57% (41.67% in Summer to 90.00% in Winter) and 3.59 (1.00 in Summer to 6.22 in Winter) respectively. Additionally, prevalence and mean intensity of infection was calculated per seasons, host size and sex. The highest values for prevalence and intensity of infection were found in winter for *P*. *homoion*. To our knowledge, this is the first ichthyoparasitological study for *A. manyasensis* in Turkey. This is also the first record of *P*. *homoion* from this host fish and locality.

Keywords: Turkey, Manyas spirlin, *Alburnoides manyasensis*, *Paradiplozoon homoion*, Seasonality, Infection statistics, Monogenea

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Introduction

As many as 409 freshwater fish species have been reported from Turkey's inland waters, 194 of these are endemic (Çiçek *et al.*, 2018). Considering family level distribution, the largest number of endemic species (110 species) are cyprinids, which include 8 species of the genus *Alburnoides* Jeitteles, 1861.*Alburnoides manyasensis* (Turan *et al.,* 2013), commonly known as "Manyas spirlin", has a very limited distribution and occurs predominantly in tributaries of the Marmara Sea, Balıkesir Province. This study is the first ichthyoparasitological survey on this fish.

To date, 6 diplozoid species have been reported from Turkey, 5 species of the genus *Paradiplozoon* (*Paradiplozoon homoion* (Bychowsky and Nagibina, 1959), *Paradiplozoon megan* (Bychowsky and Nagibina, 1959) Achmerov 1974, *Paradiplozoon bingolense* (Civáňová *et al.,* 2013), *Paradiplozoon bliccae* (Reichenbach-Klinke, 1961) and *Paradiplozoon barbi* (Reichenbach-Klinke, 1961) and from the genus *Diplozoon* there is *Diplozoon paradoxum* Von Nordmann 1832 (Öktener, 2015; WoRMS, 2020). Until 2000, morphological charactereristics were predominantly used for description and identification of diplozoid species, thereafter molecular characterization supplement morphology (Matejusová *et al.,* 2001, Civáňová *et al.,* 2013, Avenant-Oldewage *et al.,* 2014, Dos Santos *et al.,* 2015, Dos Santos and Avenant-Oldewage 2016; Dos Santos and Avenant-Oldewage, 2020). Most of the diplozoid species recorded from Turkey, with the exception of *P. barbi,* have been studied using molecular techniques and can be readily identified using available genetic data. This study presents the first report of the molecular characterization of *P. homoion* collected from Turkey, though this species is well known and studied from other localities.

Paradiplozoon homoion was previously reported in Turkey from flower fish, common carp, bleak, roach, bream, white bream and rudd (Koyun and Altunel, 2007, Soylu, 2007, Soylu and Emre, 2007, Öztürk, 2011, Akmirza and Yardimci, 2014,Öztürk and Özer, 2015, Yardımcı *et al.,* 2018). However, this study provides the first record of *P. homoion* from the endemic *A. manyasensis*. This is also the first record of the parasitic helminth fauna of *A. manyasensis* in the Nilüfer stream. Information about preference regarding season and host variables (size and sex) is also provided.

Materials and methods

Forty-six individuals of *Alburnoides manyasensis* were collected from Nilüfer stream (Bursa) between Winter 2017 and Autumn 2018, with seasonal intervals. Fish were collected from the stream by electrofishing. During each sampling effort, 10 to 12 *A. manyasensis* specimens were collected. The fish were transferred to plastic

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containers with stream water and were immediately transported to the research laboratory. Fish were kept in 20L aerated aquaria and examined within 2 to 3 hours of collection. They were killed by severing the spinal cord posterior to the cranium, then measured and divided into two groups based on length, the first group 2.00 to 7.00 cm and second group 7.10 to 12.00 cm. The sex of each fish was determined during dissection; 22 were female and 24 male. All internal organs, gill filaments, eyes, fins and the body surface were examined for parasites using a stereomicroscope. One monogenean species was collected from the gills and either prepared using glycerin ammonium picrate (Malmberg, 1957) or preserved in absolute ethanol. Data on the host fish was categorized according to the season of collection and host length and sex. Prevalence and intensity of infection were calculated following Bush *et al.* (1997). Data were analyzed using SPSS v. 25 to determine the significance of trends and differences among variables.

Morphological identification of the monogenean species was done following Khotenovsky (1985) and confirmed with DNA analysis. Genomic DNA was extracted from one individual using a NucleoSpin® Tissue kit (Macherey-Nagel, Germany) following the manufacturer's protocols. A fragment of ITS2 rDNA was amplified using primers D (5'-GGCTYRYGGNGTCGATGAAGAACGCAG-3') and B1 (5'-GCCGGATCCGAATCCTGGTTAGTTTCTTTTCCT-3'), according to reaction conditions of Matejusová *et al.* (2001). Successful amplification was verified in 1% GelRed (Biotuim) impregnated agarose gel and amplicons sequenced using BigDye v3.1 chemistry (Applied Biosystems) following Avenant-Oldewage *et al.* (2014). Electropherograms were inspected and edited manually (if required) using Geneious R6 (Kearse *et al.*, 2012) and run through the BLAST (NCBI) database (Altschul *et al.*, 1990) to identify the species.

Results

Based on the morphology observed for the collected parasite specimens, the worms were identified as *Paradiplozoon homoion*. The generated genetic data was identical to sequence data currently available from GenBank for *P. homoion* (accession numbers KP340972, AJ300715 and AF369760), confirming the identification of the worms.

Out of 46 *A. manyasensis* examined 32 were infected with 115 parasite specimens. Infection variables were recorded as follows: prevalence 69.57%, mean intensity 3.59, and abundance 2.50 (Table 1). The parasites were collected in four different seasons and thus the infection variables were also calculated per season, as per Table 1. The highest prevalence and intensity were recorded in winter, with the lowest values observed in summer. The significance of the differences in

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number of parasites collected per season were calculated using a Mann-Whitney U test, which indicated that there was significant difference between infection in summer comparedto winter (p<0.001, U=8.50) and spring compared to winter (p=0.001, U=13.00).

Table 1: Prevalence, abundance and mean intensity values of *Paradiplozoon homoion* in *Alburnoides manyasensis* from Nilüfer stream, Bursa, Turkey for four different seasons.

Infection parameters	Winter 2017	Spring 2018	Summer 2018	Autumn 2018	Total
Number of fish examined	10	12	12	12	46
Number of infected fish	9	8	5	10	32
Prevalence (%)	90.00	66.67	41.67	83.33	69.57
Abundance	5.60	1.17	0.50	3.25	2.50
Mean intensity	6.22	1.38	1.00	2.80	3.59
Maximum intensity	10	5	2	10	-

The prevalence and intensity levels of *P*. *homoion* according to length and sex of the host fish are presented in Table 2. Higher prevalence was observed in larger fish (75.00%) compared to those in small size class (57.14%). Similarly, the mean intensity reached its maximum level (4.21) in large size classes. According to Kendall's Tau test, the variables of host size and number of parasites was strongly positively correlated (p=0.009, τ=0.29). Prevalence level of *P. homoion* was higher in females (77.27%) than males (60.00%), whereas the mean intensity was higher in males (4.92) compared to females (3.18). However, the difference were not significant based on Mann-Whitney U test (p=0.898; U=215.00).

Table 2: Prevalence and intensity values of *P. homoion* in *A. manyasensis* from Nilüfer stream according to host length and sex.

	Number of fish examined	Number of infected fish	Prevalence (%)	Mean intensity	Maximum intensity
Fish length (cm)					
2.00-7.00	14	8	57.14	1.75	5
7.10-12.00	32	24	75.00	4.21	10
Fish sex					
Female	22	17	77.27	3.18	10
Male	20	13	60.00	4.92	10
Juvenile	4	2	50.00	1.00	1

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Discussion

Only *P. homoion* occurred throughout the study period. In Turkey this parasite is reported from bleak, *Alburnus alburnus*(Koyun, 2001;Koyun and Altunel, 2007), Danube bleak, *Alburnuschalcoides* and roach, *Rutilus rutilus* (Öztürk 2005, 2011), flower fish, *Pseudophoxinus antalyae* and common carp, *Cyprinus carpio*(Soylu, 2007, Soylu and Emre, 2007, Aydogdu *et al.*, 2009) and mosul bleak, *Alburnus mossulensis*(Tunç and Koyun, 2018). Detailed data related to *P. homoion* occurring in Turkey, as per the above-mentioned authors, are given in Table 3.

Table 3: Prevalence and mean intensity values of *Paradiplozoon homoion* in different host fishes from Turkey.

Host	Prevalence (%)	Mean intensity	Authors	City	Date
A. alburnus	30.2		Koyun	Kütahya (Turkey)	2001
R. rutilus	10.4		Öztürk	Bursa (Turkey)	2005
A. chalcoides	6.2		Öztürk	Bursa (Turkey)	2005
P. antalyae	73.6	8,1±0,4	Soylu and Emre	Antalya (Turkey)	2007
C. carpio	1.3	-	Soylu and Emre	Antalya (Turkey)	2007
P. antalyae	54.6	-	Soylu	Antalya (Turkey)	2007
A. alburnus	30.0		Koyun and Altunel	Lake Enne (Turkey)	2007
C. carpio	54.4	-	Aydogdu et al.	Bursa (Turkey)	2009
R. rutilus	5.4	0±0	Öztürk	Balıkesir (Turkey)	2011
A. mossulensis	19.8		Tunç and Koyun	Bingöl (Turkey)	2018

Prevalence levels recorded in the present study (69.57% , 32 out of 46 fish examined) were similar compared to findings of Soylu and Emre (2007) who reported 73.6% prevalence of *P. homoion* infecting *P. antalyae* from Kepez, Antalya.

In terms of seasonal variation, the highest prevalence was recorded in winter, while it was lowest in summer (Table 2). Influence of season on infection level of this species was investigated by some of the above mentioned records (Öztürk, 2005;Koyun and Altunel, 2007; Soylu, 2007; Soylu and Emre, 2007; Öztürk, 2011; Tunç and Koyun, 2018). Soylu (2007) reported that prevalence of *P. homoion* was highest in flower fish in winter (79.3%) and lowest in spring, corroborating the findings presented here.

In the present study, prevalence and mean intensity of *P. homoion* was highest in larger fish with a range of 7.10-12.00 cm (Table 3). The

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relationship between the level of *P. homoion* infection and size of fish is studied by Koyun (2001), Soylu (2007) and Öztürk (2011). All of these authors found a negative correlation between the infection level of *P. homoion* and host length in contrast to our findings. They postulated that infection may be related to the inability of small fish to resist parasites resulting in more parasites. We suggest that *P. homoion* infection increased with the size of hosts as larger fish provide more space for parasite attachment as suggested by Koyun and Altunel (2007), Tekin-Özan *et al.* (2008), Kurupınar and Öztürk (2009), and Aydogdu *et al.* (2014, 2015). It may also be possible that *P. homoion* opportunistically infected *A. manyasensis* which has not yet developed resistance to the parasite as the fish mature. This may indicate a recent colonization of this host species by the diplozoid.

Paradiplozoon homoion infection with respect to sex was highest on females for prevalence but higher on males for mean intensity (Table 2). On the other hand, Tunç and Koyun (2018) claim the opposite as they found higher *P. homoion* infections in male fish. This may indicate that the males of *A. mossulensis* and the females of *A. manyasensis* have similar behavior or feeding habits.

In conclusion, the present study is the first parasitological survey of *A. manyasensis,* which found to be infected by *P. homoion*. It is therefore a new parasite species record for the host fish in Turkey and a new locality for distribution of *P. homoion*. Additionally, the present study adds some valuable information on seasonal occurrence and the physical parameters (length and sex) of the host on infection variables of the parasite.

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