Journal of Limnology and Freshwater Fisheries Research

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The First Ectohelminth Ichthyo-Parasitic Fauna of the Turkish Endemic Fish, Marmara Barbel, *Barbus oligolepis* Battalgil, 1941, with New Host and Geographical Record

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ABSTRACT

The aim of this study was to determine the ectoparasite helminth fauna of Turkey endemic fish, Barbus oligolepis (Battalgil, 1941), from Susurluk Stream in the village of Yıldız, Balıkesir. For this purpose, a total of 81 individuals of *B. oligolepis* were caught seasonally by electrofishing between the 2020 spring (April) and 2021 winter (February) and the ectoparasite helminth fauna was studied. Only two monogenean species, *Dactylogyrus carpathicus* (Zakhvatkin, 1951) and *Paradiplozoon homoion* (Bychowsky and Nagibina, 1959), were identified. As a result of this examination *P. homoion* was the most prevalent and the highest number in the host fish. This species was present throughout all seasons. A total of 126 specimens of *P. homoion* infected 29 of the 81 fish examined, with a prevalence and mean intensity of 35.8 (1.5 %), parasite/fish respectively. Additionally, infection parameters for two monogenean species were calculated in accordance with the season, host size and sex. This study is the first to report the presence of an ectoparasitic helminth for B. oligolepis in this location in Turkey. *P. homoion* and *D. carpathicus* are also new ectoparasitic helminth records from this host fish and location. In addition, sequence data of P. homoion from host fish were reported to GenBank for the first time with this study. As a result, new information about the geographical distribution and host range of two parasite species has been added.

Keywords: Platyhelminthes, Monogenea, *Dactylogyrus carpathicus*, *Paradiplozoon homoion*

ARTICLE INFO

RESEARCH ARTICLE

Received : 04.01.2023
Revised : 20.04.2021
Accepted : 07.03.2023
Published : 25.12.2023

DOI:10.17216/LimnoFish.1226325

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Türkiye Endemik Balıklarından Marmara Bıyıklısı, *Barbus oligolepis* Battalgil, 1941' in Yeni Konak ve Coğrafik Kayıt ile İlk Ektohelmint İhtiyo-Parazitik Faunası

Öz: Bu çalışmanın amacı, Balıkesir ili Yıldız Köyü Susurluk Deresi'nden *B. oligolepis* 'in ektoparazit helmint faunasının belirlenmesidir. Bu amaçla 2020 ilkbahar (Nisan) ile 2021 kış (Şubat) tarihleri arasında mevsimsel olarak elektroşoker ile toplam 81 *B. oligolepis* bireyi yakalanmış ve ektoparazit helmint faunası çalışılmıştır. Bu inceleme sonucunda sadece iki monogenean türü *Dactylogyrus carpathicus* ve *Paradiplozoon homoion* tespit edilmiştir. *P. homoion'* un konak balıklarda en yüksek yaygınlıkta ve en fazla sayıda olup, her örnekleme mevsiminde tespiti yapılmıştır. Toplam 129 *P. homoion* bireyi İncelenen 81 balık bireyinin 29'unu %35,8 enfeksiyon yaygınlığı ve 1,5 parazit/balık ortalama yoğunluk ile enfekte ettiği belirlendi. Bunlara ilaveten, iki monogenean türün enfeksiyon parametreleri mevsim, konak balık boy ve cinsiyet grupları açısından değerlendirilmiştir. Bu çalışma, Türkiye'de Susurluk Çayı'ndaki *B. oligolepis* için ektoparazitik helmint varlığını bildiren ilk çalışmadır. *P. homoion* ve *D. carpathicus* da bu konak balıktan ve bölgeden yeni ektoparazitik helmint kaydıdır. Ayrıca konuk balıklardan alınan *P. homoion*'un sekans verileri ilk kez GenBank'a bu çalışma ile bildirildi. Sonuç olarak, iki parazit türünün coğrafi dağılımına ve konak yelpazesine yeni bilgiler eklenmiştir.

Anahtar kelimeler: Plathelminthes, Monogenea, *Dactylogyrus carpathicus*, *Paradiplozoon homoion*

How to Cite
Aydoğdu N, Torcu Koç H. 2023. The First Ectohelminth Ichthyo-Parasitic Fauna of the Turkish Endemic Fish, Marmara Barbel, *Barbus oligolepis* Battalgil, 1941, with New Host and Geographical Record 9(3): 130-137. doi: 10.17216/LimnoFish.1226325.

Introduction

In Türkiye, the genus *Barbus* has 12 valid species of Cyprinid fishes. As well as *Barbus oligolepis,* at least five species are endemic to Türkiye (Çiçek et al. 2020). *B. oligolepis* is known to inhabit the following rivers that drain to the southern shore of the Marmara Sea in Türkiye: namely, Kocasu, Nilüfer River; Kocaçay, Susurluk River; Hançay, Gönen and Narlıca Stream, İznik (Turan et al. 2009). Generally, this species habits in swift-flowing water, with a stone and pebble bottom (Froese and Pauly 2022).

To the best of our knowledge, only two studies have investigated the presence of helminth parasites in endemic fish species of the genus *Barbus* in Turkey. Aydogdu et al. (2017) studied helminth parasites of *B. niluferensis* (Turan et al. 2009) from the Nilüfer stream*;* similarly, Önalan et al. (2022) studied the endohelminth parasites of *Barbus ercisianus* (Karaman, 1971) from the Nemrut Crater Lake**.** However, no ichthyo-helminthological studies have been performed for *B. oligolepis* so far. As a result, this study represents the first ichthyo - helminthological survey of helminth parasites in this endemic fish in Türkiye. In addition, various studies have been conducted on the helminth fauna of native fish species of the genus *Barbus* in Turkey (see, for example, Koyun 2001; Develi 2008; Turgut et al. 2011; Turgut and Özgül 2012; Koyun et al. 2015). Only three of these studies Develi (2008), Turgut and Özgül (2012), Koyun et al. (2015), were able to record the ectoparasitic helminth species parasitizing on *Barbus* spp.

In the studies mentioned above, ectoparasitic helminth species parasitized in this genus are represented in three genera, *Dactylogyrus*, *Gyrodactylus* and *Dogielius*. They reported on the occurrence of ectoparasitic helminth species in native fish species of the genus *Barbus* in Türkiye, including, *Dactylogyrus goktschaicus* (Gussev, 1966), *D. lenkorani (Mikhailov, 1967), Dactylogyrus malleus* (Linstow, 1877*), Gyrodactylus elegans* (von Nordmann, 1832), *Gyrodactylus hemibarbi (Ergens, 1980), Gyrodactylus* sp. and *Dogielius mokhayeri* (Jalali and Molnar,1990*)*. In addition to these, no previous study has found *Paradiplozoon* spp. in fish species of the genus *Barbus*. Therefore, the present study is the first in Turkey to present data on diplozoid species in *Barbus* spp. and to report sequence data to GenBank.

So, the current study aimed to isolate, identify and provide additional information on the ectoparasitic helminth species of *B. oligolepis* from freshwater ecosystems of the Susurluk stream in Türkiye, as well as to determine their dependence on the season, host sex and host length. Thus, it will contribute to increasing the diversity of ectoparasitic helminth species recorded in *Barbus* spp. in previous studies. As a result of this investigation, we will provide the first records of the ectoparasitic helminth of host fish for Türkiye as well as contribute to our understanding of the geographical distribution and host range of the parasite species identified from the host fish.

Materials and Methods

Overall, 81 individuals of Marmara barbell, *Barbus oligolepis* were collected from Susurluk Stream, the village of Yıldız, Balıkesir ($39^0$48’973’’K and $28^0$10’14’’ D) between spring 2020 (April) and winter 2021 (February), with seasonal intervals (Figure 1). Fish were collected from the stream by electrofishing. The individuals were placed in plastic containers filled with stream water and immediately transported to the research laboratory as live subjects. They were kept in aerated fish tanks in the laboratory and necropsied for ectoparasitic helminth approximately 3-4 hrs after collecting. Dissections were performed within 3-4 hours. The fish were killed by severing the spinal cord behind the head, and their total length were measured and divided into three groups based on length, to the nearest 10 cm. During dissection, the sex of each fish individual was determined; 40 females and 41 males. During dissection, all internal organs, gill filaments, eyes, fins and the body surface of each fish individual were examined for ectoparasitic helminth under an Olympus stereomicroscope with 16x-40x magnification. Two monogenean species were collected from the gills and either prepared using glycerin ammonium picrate or stored in ethanol solution (Malmberg 1957). Data on each fish individual was categorized according to seasons, host fish size and sex groups. Morphological identification of the monogenean species was performed using the identification keys developed by Gussev (1985, 1987), Khotenovsky (1985) and available references; Prevalence, intensity and abundance of infections were calculated according to Bush et al. (1997) and the mean and standard deviation of each parameter was determined using Microsoft Excel (Office 2000). Kruskal-Wallis (more than two groups) tests were applied to find significant differences in the mean intensity of the parasite species for host fish size and seasons. The Mann-Whitney U test (two groups) was used to determine the correlation between the intensity of each helminth species infection and the host sex. The significance level of $\alpha \leq 0.05$ was used. All statistics analyses were performed using SPSS v. 23.

Furthermore, the molecular analysis was performed to confirm the morphological identification of diplozoid species as previously described by Aydogdu et al. (2020a, 2020b).

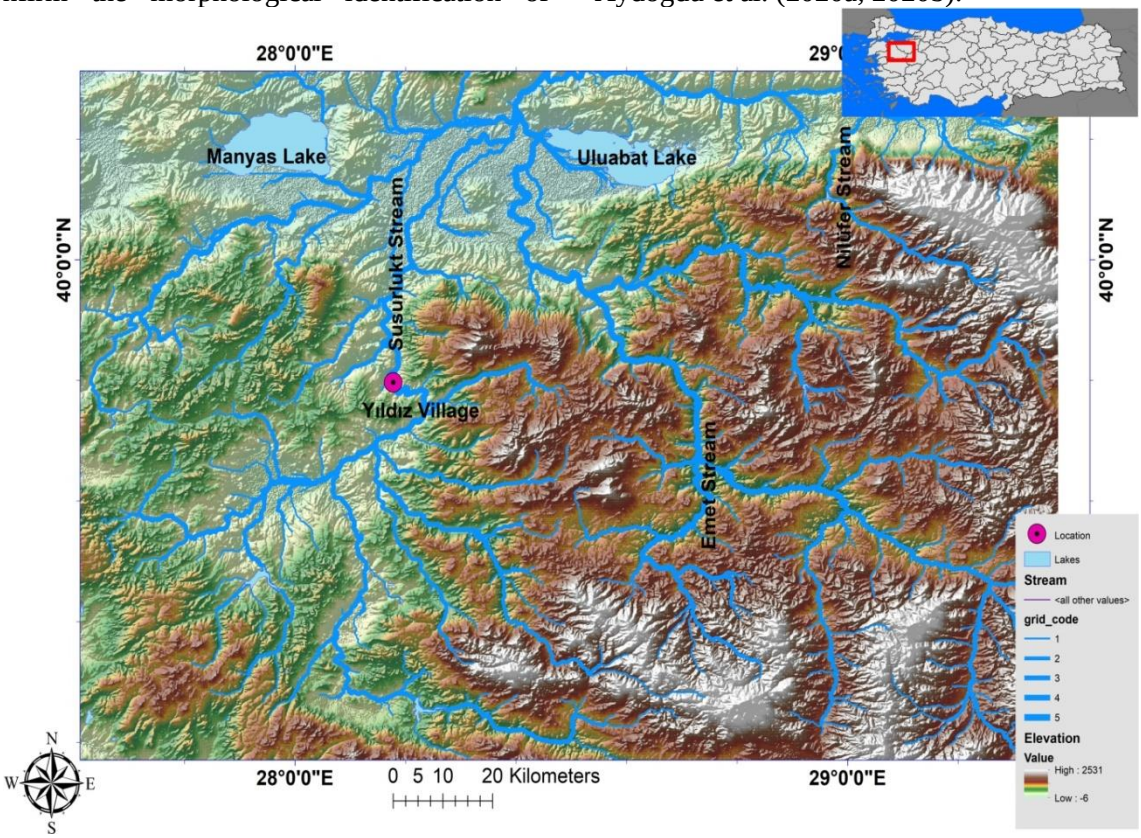

Figure 1. Sampling locality of *B. oligolepis* in the Susurluk Basin Map

Results

This study investigated ectoparasites of *Barbus oligolepis* in Susurluk Stream (Balıkesir) between the 2020 spring and 2021 winter. This examination revealed that 45 of 81 fish (55.5%) were infected with one or two monogenean species. According to the morphology observed in the collected parasite samples, only two monogenean species were identified, i.e., *Dactylogyrus carpathicus* (Figure 2, 3 and 4) and *Paradiplozoon homoion* (Figure 5) and we also confirmed *P. homoion* based on molecular analysis (Genbank assession number: OP558585).

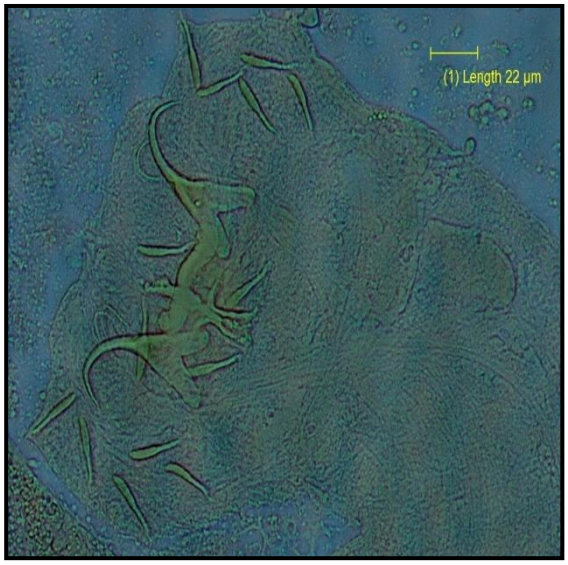

Figure 2. *D. carpathicus* haptor

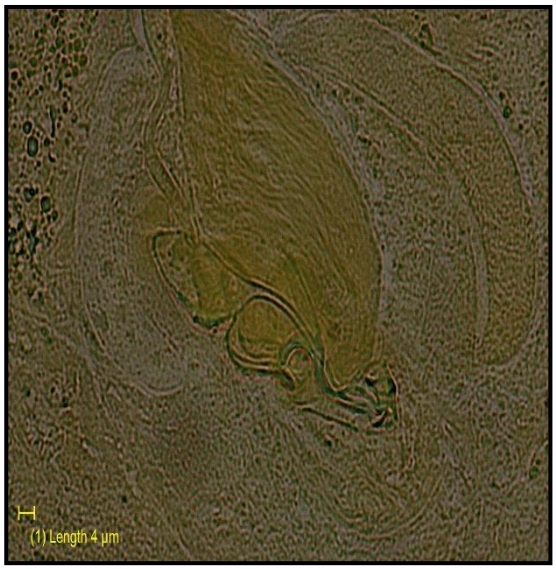

Figure 3. *D. carpathicus* copulatory organ

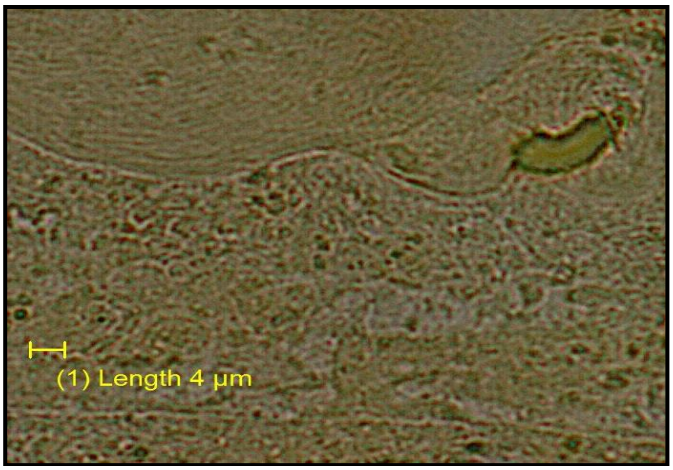

Figure 4. *D. carpathicus* vaginal tube

In our study, *P. homoion* (Figure 5) was the most prevalent and the highest number in the host fish. Moreover, it was detected in every sampling season. A total of 126 specimens of *P.homoion* (Figure 5) were found on 29 of the 81 fish examined. Infection variables were recorded as follows: prevalence 35.8 %, mean intensity 4.3 parasite/fish and mean abundance 0.6 (Table 1).

Figure 5. Structure of *P. homoion'*s clamps

Table 1. Distribution of infection value of ectohelminth parasites in *B. oligolepis* from Susurluk Basin, Balıkesir

Parasite species	Number of fish Infected	No. of parasites collected	Prevalence (%)	Mean intensity	Abundance
D. carpathicus	22	71	27.1	3.2	0.8
P. homoion	29	126	35.8	4.3	1.5

Seasonal prevalence of infection was highest in spring, 73.6 % ($p=0.016$, $p>0.05$). The highest abundance is also found in the spring, while the highest mean intensity (parasite/fish) was found in the winter season (Table 2).

Table 2. Distribution of infection value of ectohelminth parasites in *B. oligolepis* from Susurluk Basin, Balıkesir, according to seasons

Name of Parasites	Infection Parameters	Seasons			
		Spring 2020 (n: 19)	Summer 2020 (n: 20)	Autumn 2020 (n: 22)	Winter 2021 (n:20)
D. carpathicus	Infected Fish	10	3	9	
	Prevalence (%)	52.6	15	37.5	
	Mean Intensity	4	6.3	1.3	
	Abundance	2.1	0.9	0.5	
	Total parasite no	40	19	12	
P.homoion	Infected Fish	14	5	4	6
	Prevalence (%)	73.6	25	18.1	30
	Mean Intensity	4.5	3.4	3.7	5
	Abundance	3.3	0.8	0.6	1.5
	Total parasite no	64	17	15	30

A total of 71 specimens of *D. carpathicus* were recovered from 22 of 81 individuals of *B. oligolepis* examined (prevalence 27.1 %, mean intensity 3.2 parasite/fish, mean abundance 0.8 respectively) (Table 1). Seasonal prevalences varied between 15 and 52.6 %, highest in spring and lowest in summer and mean intensities of 1.3 to 6.3, highest in summer, varied significantly (Kruskal-Wallis p=0.135, p>0.05). The mean abundance of 0.5 to 2.1 was also highest in spring. This species was not found in any of the winter samples (Table 2).

The prevalence, intensity and abundance levels of *P. homoion* and *D. carpathicus* according to the size of the host fish are presented in Table 3. Both monogenean species were found in all fish size classes and infection values varied according to fish size classes (Table 3).

P. homoion was found on fish of all size classes, with prevalences varying between 27.2 and 44.4 %. The highest infection levels occurred in size class III (44.4 %) and its highest mean intensity levels were recorded in size class III (5.5 parasite/fish). However, there were no statistically significant differences between the size groups (p=0.726, p>0.05) in terms of the abundance level of this species. For *D. carpathicus*, the lowest prevalence was in size class I (15.3%), while the highest rate was in size class II (39.3%). Mean abundance of 0.2 to 1.6 was found to be highest in size class II (Table 3).

Table 3. Distribution of infection value of ectohelminth parasites in *B. oligolepis* from Susurluk Basin, Balıkesir, according to the host length

Fish Classes Groups (cm)	Parasite species	Infected Fish	Prevalence (%)	Mean intensity	Abundance	Total parasite no
10-20 (n=39)	*D. carpathicus*	6	15.3	1.6	0.2	10
	P. homoion	16	41	3.8	1.5	62
20.1-30 (n=33)	*D. carpathicus*	13	39.3	4	1.6	53
	P. homoion	9	27.2	4.6	1.2	42
30.1< (n=9)	*D. carpathicus*	3	33.3	2.6	0.8	8
	P. homoion	4	44.4	5.5	2.4	22

There were no statistically significant differences in abundance levels of this species between the size classes of the host fish (p=0.135, p>0.05). The prevalence, intensity and abundance levels of two monogenean species according to the sex of the host fish are presented in Table 4. For *P. homoion*, prevalence and mean intensity levels were both higher in females (37.5%, five parasite/fish) than in males (34.1%, 3.5 parasite/fish). The maximum parasite number was found in female fish specimens for this parasite species (Table 4). However, there was no statistical correlation between the numbers of *P. homoion* parasites between sex groups of the host fish (p=0.526, p>0.05). The prevalence and intensity levels of infection of *D. carpathicus* were both higher in females (30%, 3.5 parasite/fish) than in males (24.3%, 2.9 parasite/fish). Similarly, *D. carpathicus* was recorded in maximum numbers in female individuals of the host fish. In contrast to these findings, no correlation was found between the abundance of *D. carpathicus* and host sex (p=0.627, p>0.05).

Table 4. Distribution of infection value of ectohelminth parasites in *B. oligolepis* from Susurluk Basın, Balıkesir, according to the host sex

Fish Sex Groups	Parasite species	Infected Fish	Prevalence (%)	Mean intensity	Abundance	Total parasite no
Female (n=40)	*D. carpathicus*	12	30	3.5	1	42
	P. homoion	15	37.5	5	1.9	76
Male (n=41)	*D. carpathicus*	10	24.3	2.9	0.7	29
	P. homoion	14	34.1	3.5	1.2	50

Discussion

This study investigated ectohelminth ichthyo-parasitic fauna of the endemic fish, *Barbus oligolepis*, from Susurluk stream, the village of Yıldız, Balıkesir. Only two monogenean species were identified, namely *Dactylogyrus carpathicus* and *Paradiplozoon homoion*. In the present study, *D. carpathicus* were identified using morphological and anatomic assessment. The identifying feature of this species, which separate it from other closely related species in the genus *Dactylogyrus*, considered during the current study was that the anchors have nearly equally sized rots (dorso and ventro apical length), quite characteristic male copulatory organs, vaginal tube and ventral bars. (Figure 1, 2). Besides, the measurements of chitinoid structure parts served as the basis for identifying the species.

In the case of *P. homoion*, we described it based on morphology and confirmed it using molecular analysis. In addition to these, this is the first survey on the ichthyo-helminthological data for *B. oligolepis* in Türkiye. Furthermore, the host fish is the new host record for the two monogenean species. Moreover, *D. carpathicus* and *P. homoion* were found for the first time at Biotope Yıldız. Therefore, the present study adds new data to the geographical distribution and the host range of two parasite species. To date, ectoparasitic helminth species parasitizing freshwater fish in Turkey are mostly represented in two genera: *Dactylogyrus* and *Gyrodactylus*. *Dactylogyrus*, with 47 species, is the genus that representing the highest number of species parasitized in freshwater fish in Türkiye (Özer 2021). On the other hand, parasitic individuals of this genus recorded in 14 different freshwater fish species living in different habitats in Türkiye were not defined at the species level (Özer 2021). *D. carpathicus*, which we recorded in our current study, is one of the species defined at the species level. So far, *D. carpathicus* has only been reported from the Doğancı Dam Lake and Nilüfer Stream in the Marmara region in Türkiye (Aydogdu et al., 2002; Aydogdu and Kubilay, 2017). Therefore, the present study adds new data to the geographical distribution and the host range of *D. carpathicus*.

In the studies mentioned above, these authors have also investigated the effect of season on infection levels of this species. In this study, the highest infection levels of D. *carpathicus* in spring (52.6%) are similar to the findings of Aydogdu et al. (2002) in Doğancı Dam Lake. Aydoğdu and Kubilay (2017) found that the prevalence of infection with this species was highest in winter (92.3%) in *Barbus niluferensis* in the Nilüfer stream in Turkey's north west region. It is followed by the spring season, which has an 85.7 % prevalence. These findings are inconsistent with ours.

According to Özer (2021), the family Diplozoide consists of three genera: *Diplozoon*, *Eudiplozoon* and *Paradiplozoon*. To date, these three genera include eight named species of parasites and five parasites that have not been identified as a species in Türkiye. Among these genera, the genus *Paradiplozoon* is represented by the most species with six named parasite species in Turkey. Among these species, *P.*

homoion is the most recorded species so far in Turkey (see, for example, Aydogdu et al. 2020a, 2020b). According to these studies, this is the first record of *P. homoion* from this host fish and locality. Therefore, the present study increases the number of host fish and localities where *P. homoion* has been recorded in Türkiye.

The seasonal variation of the infection of *P. homoion* was also investigated in our study. The highest infection prevalence of this species was recorded in spring (73.6%) (Table 2). Infection intensity of the parasite peaked in winter (5 parasites/fish) (Table 2). Seasonal variation of *P.homoion* infection rates has also been studied in different fish species in Turkey so far (Koyun 2001; Öztürk 2005; Soylu 2007; Aydogdu et al. 2020a, 2020b). Aydogdu et al. (2020a, 2020b) from Manyas spirlin, *Alburnoides manyasensis* from Nilüfer stream, Bitterling fish, *Rhodeus amarus* (Bloch 1782) from Susurluk Stream, respectively and Soylu (2007) from flower fish, *Pseudophoxinus antalyae* (Bogutskaya, 1992) from Kepez, Antalya studied in seasonal variation of infection rates of this species. They recorded the highest infection prevalence value of *P. homoion* in the winter season. Contrary to these findings, Koyun (2001) did not find this species from bleak in winter, *Alburnus alburnus* (Linnaeus 1758). Similarly, while Öztürk (2005) recorded *P. homoion* from *Rutilus rutilus* (L., 1758) in all seasons except winter, the same researcher found this species only in *Chalcalburnus chalcoides* (Güldenstaedt, 1772) in summer in the same study. The findings of our study are similar to the findings of the studies mentioned above before our study. According to the authors, this situation could be due to different rates of parasite development in different waters. We also support this view.

Regarding the connection in this study, the highest prevalences and mean intensities of *D. carpathicus* were observed in fish 20.1-30 cm long (Group II) (Table 3), indicating a link between the infection level of two ectoparasitic helminth species and host fish size. The relationship between the infection levels of this species and host fish sizes in Türkiye was studied only by Aydogdu and Kubilay (2017). These authors investigated the relationship between infection rates of *D. carpathicus* and host length in *Barbus niluferensis.* In contrast to our study for *D. carpathicus*, they found the highest mean intensity of infection in the fish size classes of 15.1-20 cm (Group II) despite recording the highest prevalence levels of infection in the fish size classes of 10-15 cm (group I), *P. homoion*, was found in fish of all size classes. Infection prevalence levels and mean density for *P. homoion* were highest in the 30.1 < cm fish size classes (group III), (Table 3). According to current data, only four ichthyoparasitological studies in Türkiye have been conducted to determine the relationships between infection levels of *P. homoion* and host fish sizes Koyun (2001), Soylu (2007), Öztürk (2011), Aydogdu et al. (2020a). Among these four different studies, only Aydoğdu et al. (2020a) observed the highest prevalence levels of infection of this species in large-size classes (7.1- 12 cm). This study lends support to the findings of our study in this context.

In our study, the prevalence and abundance of *D. carpathicus* and *P. homoion* were higher in the female host than in the male host. However, there was no statistically significant difference in the number of *D. carpathicus* (p=0.627) and *P. homoion* (p=0.526) between the sexes of the host fish. In this context, in only one Aydogdu and Kubilay (2017) of the two studies in which *D. carpathicus* was found in our country, the preference of parasite infection values of this species based on sex was investigated and the prevalence, the mean intensity values of infection in female fish were found to be higher. Our study is in line with the findings of Aydogdu and Kubilay (2017). As for *P. homoion*, the highest prevalence and mean intensity levels of infection were the highest in female fish (Table 4). In their study, Aydogdu et al. (2020b) found the highest prevalence and mean intensity levels of this species in female fish in *Alburnoides manyasensis* (Turan et al. 2013). The results of the present study also confirm the finding of Aydogdu et al. (2020b)**.** In contrast to our study, Tunç and Koyun (2018) recorded the highest infection rate of this species in male fish.

The purpose of this study was to determine the ectoparasitic helminth fauna of Türkiye endemic fish, *B. oligolepis*, from the Susurluk Stream, in the village of Yıldız, Balıkesir. As a result of the conducted study of 81 individuals of *B. oligolepis*, only two monogenean species *D. carpathicus* and *P. homoion* were identified. Among these parasites, *P. homoion* was the most prevalent and had the highest number in the host fish. To our knowledge, *D. carpathicus* and *P. homoion* are the first records of this host fish. Additionally, Susurluk Stream was identified as a new locality record for two monogenean species. Furthermore, in the present study, the prevalence, mean intensity and abundance of helminth parasites were calculated in accordance with season, host fish length classes and sex. As a result, new information about the geographical distribution and host range of these helminth species has been gained.

Conflict of interest

The authors declare no conflict of interest

Compliance with ethical standards

During the study, no treatment/experiment was implemented on the live animal. All sampling and laboratory work on fish complies with the Republic of Turkey, Ministry of Agriculture and Forestry animal welfare laws.

Data availability statement

No data availability statement

Acknowledgements

This paper is a part of the Doctorate Thesis named '' An Investigation On Helminthic Parasites Which Were Determined In Some Fish Species Living In Susurluk Basin''

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