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Research Article

Ectoparasitic Helminths During the Annual Cycle of the European Chub (*Squalius cii*, Richardson, 1857) in the Susurluk Basin, Türkiye: Their Infestation Levels, Identification, and Effect of Host Factors on Infection Levels*

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ABSTRACT

The ectoparasitic helminth in the European chub (*Squalius cii*, Richardson, 1857) was collected from the Susurluk basin from the Northwest region of Türkiye in the period from spring 2020 (April) to winter 2021 (February) once in every three months and also summarised. Only two species of Monogenea were found on the gills of the host and were identified as *Dactylogyrus vistulae* Prost, 1967 (Monogenea: Dactylogyridae) and *Paradiplozoon homoion* (Bychowsky & Nagibina, 1959) (Monogenea: Diplozoidae). *D. vistulae* was the most common and the highest number species. Other factors such as season, host length and sex were investigated to determine their effect on the infection values of ectoparasitic helminth infection, which were calculated from information collected from 79 host fishes. However, according to our current literature, there is no study in which *S. cii* is considered as a valid species and investigated in terms of ichtyohelminthological in Türkiye. This is the first ichtyohelminthological survey of *S. cii* in Türkiye and therefore new host and distribution records for all helminth species. Moreover, with such studies, scientists will have the opportunity to evaluate the infection success of parasite species belonging to this group, depending on the host, environmental conditions and enemies, and their infection success depending on the seasons and host factors. And data will be obtained about the complex life histories of the species belonging to this group and will contribute to the determination of the causes of death that will occur in both cultured and natural fish populations in the future.

Keywords: *Squalius cii*, *Dactylogyrus vistulae, Paradiplozoon homoion*, season, host size, sex

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INTRODUCTION

The genus *Squalius* of the family Cyprinidae has a wide geographical distribution in Europe and Asia (Özulug & Freyhof 2011).According to the current literature, this genus includes about 47 species (Zardoya & Doadrio 1999) and 21 of 47 from Central and Western Anatolia (Çiçek et al., 2020; Özuluğ& Freyhof 2011). Despite these studies, the taxonomy of these genus members in Türkiye at the species level has not yet been resolved. Indeed, in one of these studies, Özuluğ & Freyhof (2011) revised the species belonging to the genus *Squalius* in Anatolia and defined ten species. Four of them were recognised as new species and the other six as valid species. One of these valid species is*Squalius cii*, which occurs in northwest Anatolia in Türkiye and is present in all branches of the stream in the Susurluk basin (Öztürk &Küçük 2017). In addition, the taxonomic status and distribution of the species of the genus *Squalius* were also studied by Stoumboudi et al. (2006). They has been reported this species from the island of Lesbos (Greece). The studies of these researchers added a locality outside of Türkiye to the distribution area of the species. However, the taxonomic and the current status of the species

of this genus still remain complicated. In fact, while this species was considered as endemic to Türkiye by Aksu et al.(2016), Çiçek et al. (2020) were accepted as a natural species. As for the status of *Squalius cii* in the IUCN, although this species is narrowly distributed and consumed by local people in a small number of locations, it is still Least Concern (LC) in the IUCN.

Although there have been many studies investigating ichthyoparasitological properties of freshwater fishes in Türkiye in recent years, there are a few studies on freshwater fishes belonging to *Squalius* (Aydogdu et al. 2015; Elbay & Öztürk 2021; Soylu et al. 2017; Unal et al. 2017)and none on the European chub (*S. cii)*. However, according to our current literature, there is no study in which *S. cii* is considered as a valid species and investigated in terms of ichthyoparasitological. Therefore; this study is the first ichtyohelminthological survey on *S. cii* in Türkiye. *S. cii* was investigated for ectoparasitic helminth diversity and determined the effect of seasons, host fish age and sex on their infection levels.

MATERIALS AND METHODS

Between Spring 2020 to Winter 2021 (one every three months), specimens of *Squalius cii* were collected seasonally from the locality (Susurluk basin) (Fig. 1). The fish was collected by electrofishing, attempting to collect an equal number of small and medium-sized fish. A total of 79 *S. cii* were sampled and submitted for ichtyohelminthological investigation in the laboratory in within polythene tanks aerated and containing ice to minimize any possible damage from the locality and examined within as soon as possible after collection. The fish specimens were killed by vertebral separation and their length recorded. Specimens were grouped according to length. During dissection, first fins and skin were first observed with a Nikon binocular stereoscopic microscope, and thereafter, the gills of freshly killed fish were dissected into Petri dishes for careful observation for the presence of ectoparasitic helminth. The sex of individual fish were determined after dissection. The monogenea specimens were made into permanent preparations according to the methods described in Malberg 1957; Ergens, 1969. Infection parameters for each parasite species were recorded on the basis of seasons, host sex, and length. The monogenea specimens were identified according to the identification keys of Markevic (1951);Bychowkaya –Palovskaya (1962);Gussev (1985); Khotenovsky (1985). For calculations of the levels of their infection parameters values were calculated using Bush et al. (1997). Infection parameters for each monogenean species were calculated according to Bush et al. (1997). The SPSS V.23 software was applied to determine the significance between the prevalence and intensity of parasite infestation on the basis of host sex, length and seasons.Subsequently, to confirm the morphological identification of the diplozoid species, the molecular analysis was performed as reported by Aydogdu et al. (2020a, b). The BLAST sequence analysis have available in GenBank under accession number MW724525 as *Paradiplozoon homoion*.

Figure 1. Sampling locality of *Squalius cii* in the Susurluk Basin Map.

RESULTS AND DISCUSSION

General infection

Two different ectoparasitic helminths were identified on the gills of 79 individuals of *Squalius cii* examined. Based on the presence of the following morphometric characteristics: the hard parts of haptor and reproductive organs, they were identified: *Dactylogyrus vistulae* (Prost, 1967), (Fig.2a,b,c) was identified from 18 individuals and *Paradiplozoon homoion*(Bychowsky & Nagibina, 1959), (Fig. 3) from 9 individuals of *S. cii*. In total, 62 ectoparasitic helminth specimens were recovered. Among these, *D. vistulae* was the most common and the highest number species (Fig. 4). Figure 4 graphically shows the calculated infection values for two ectoparasitic helminth species.

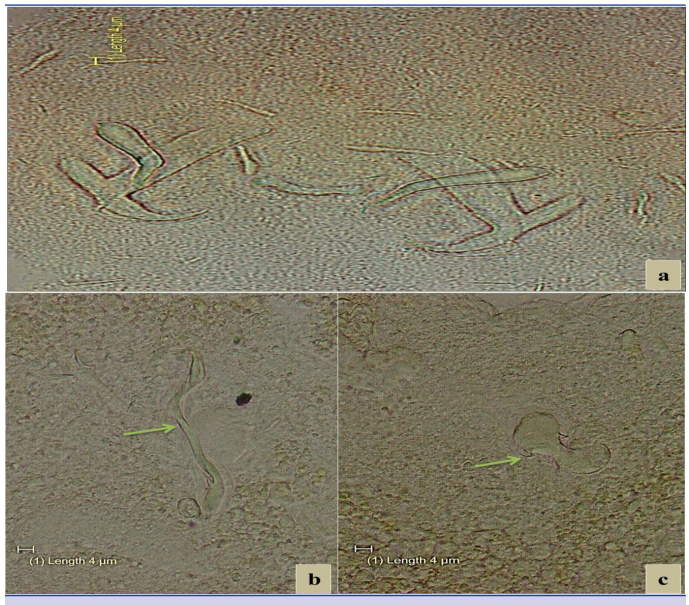

Figure 2. *Dactylogyrus vistulae* a) haptor (scale bar = 4µm) b) copulatory organ(scale bar = 4µm). c) vaginal tube (scale bar = 8µm).

Figure 3. Clamps of the *Paradiplozoon homoion* from Squalius cii (scale bar = 8µm).

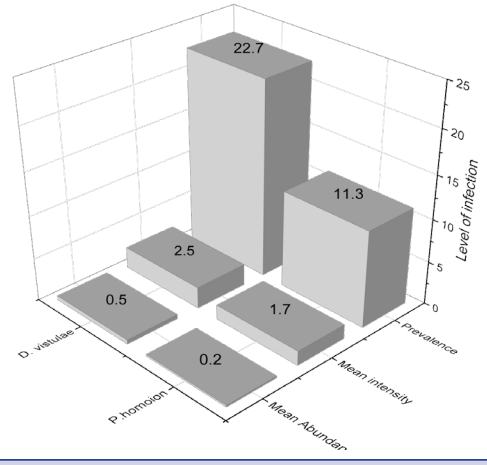

Figure 4. Distribution of the infection value of ectohelminth parasites in *Squalius cii* from the Susurluk Basin, Balıkesir.

The distribution of European chub ectoparasitic helminth with respect to season

The study was conducted seasonally from spring 2020 (April) to winter 2021 (February). *D.vistulae* was recorded in spring and summer, while *P.homoion* was observed throughout all seasons, with the exception of spring. The highest prevalence value of *D. vistulae* was recorded in spring and summer. In *P. homoion*, it was highest in winter (10%) (Fig 5).As far as seasonally intensity is concerned; *D. vistulae* had the highest mean intensity values in spring and summer, while the highest mean intensity for *P. homoion* was observed in summer. As a result of the analysis, it was determined that the parasite infection rates of *D.vistulae* changed significantly according to the seasons (p=0,000), while *P.homoion* was not found to be significant (p=0,262).

The distribution of European chub ectoparasitic helminth with respect to the host length classes

The fishes were measured in term of length with measuring scale and divided into two groups according to their length. The highest prevalence of *D. vistulae* was recorded in length classes II (11.6-16.6 cm), whereas the highest prevalence of *P homoion* was observed in fish between 6.5-11.5cm (length classes I) (Fig.7, 8). For the mean parasite intensity in the present study, the mean intensity of the ectoparasitic helminth also showed differences in the two length classes. In *D. vistulae* and *P.homoion*, the mean parasite intensity was highest in larger fish.

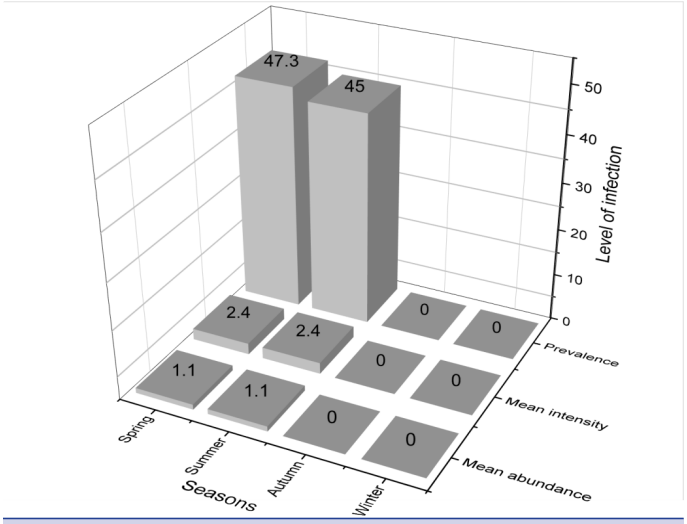

Figure 5. Presence of *Dactylogyrus vistulae* in *Squalius cii* in different seasons.

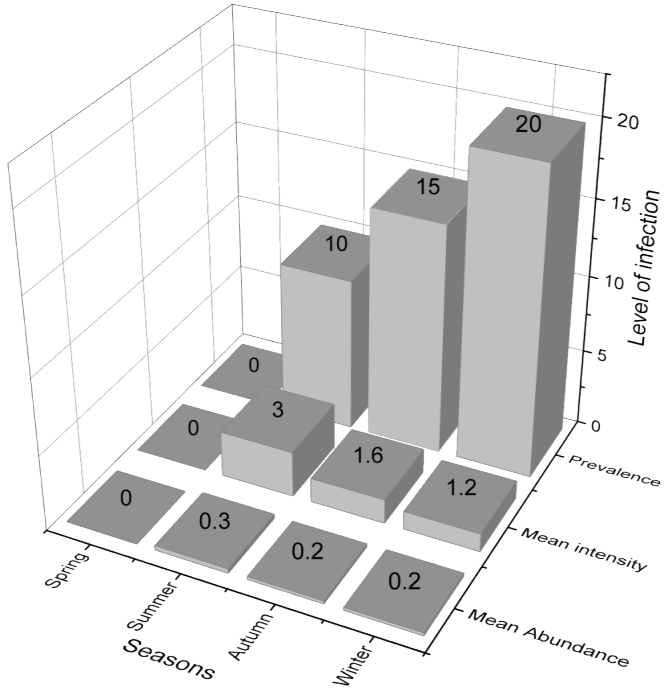

Figure 6. Presenceofthe *Paradiplozoon homoion* in *Squalius cii* indifferent seasons.

In addition, the mean abundance of ectoparasitic helminth specimens was almost equal in length classes (Fig.7, 8). The statistical analyses showed no significant difference in the number of *D. vistulae* and *P homoion* between host length classes (p=0.053 and 0.901, respectively).

The distribution of European chub ectoparasitic helminth with respect to the host fish sex

Of the 79 host *S. cii* individuals, 65.8% were females and 34.13 % were males. The highest prevalence of *D. vistulae* was in males, and the highest mean intensity level was in females (Fig. 9, 10). With respect to *P homoion* infection, the prevalence and mean intensity were both higher in females than in males. However, the difference was not significant based on the statistical analyses (p=0.307 and 0.383, respectively).

This present work provides new host records and a new locality was added for the distribution of two ectoparasitic helminth specimens. The infection values of the ectoparasitic helminth specimens varied between species. *Dactylogyrus vistulae* Prost 1967, (Fig.2a, b, c) had the highest prevalence, intensity and abundance (Fig.4). *D. vistulae* (a monogenean) is very common in fish belonging to the genera *Squalius* and *Chondrostoma* (Gussev 1995) and has been previously reported from five fish species living in different habitats from Türkiye: *Squalius cephalus*, S. *anatolicus, S. recurvirostris, Leuciscus cephalus* and *Chondrostoma regium* collected from Serban Dam Lake, Susurluk Creek, Dogancı Dam Lake, Bursa, Örenler Dam Lake, Yeşilırmak River, Almus Dam Lake and Akçay stream with prevalence and mean intensity varying from 12.04 % and 4.5 to 63.6 % and 8.5%, respectively. (Aydogdu et al. 2001; Açıkel & Öztürk 2012; Turgut et al. 2012; Öztürk 2014; Aydogdu et al. 2015; Elbay & Öztürk 2021). Thus, it can be seen that the prevalence and mean intensity of infection with *D. vistulae* differs according to the host species and locality. As a result, this study and the studies of the researchers mentioned above showed that the prevalence and mean intensity values of *D. vistulae* varied according to the host fish species and locality. The changes in the prevalence and mean intensity values of *Dactylogyrus* spp. infection in freshwater fishes might be influenced by various factors: such as host specificity, host hormonal status, host immunological response, host migration …etc. (Ramadan &Shakweer 1981; Hanzelova & Zitnan 1985; Tombi et al. 2004; Simkova et al. 2005; Açıkel& Öztürk 2012;Koyunet al. 2015).However, the influence of these factors is difficult to distinguish because they are likely interrelated and affect each other.

In the present study, a total of 16 specimens of *Paradiplozoon homoion* (Bychowsky & Nagibina 1959), (Fig. 3) were recovered from 9 of 79*S. cii* examined with prevalence and mean intensity of 11.3% and 1.7 %, respectively. It is also the most recorded species of *Paradiplozoon* in Türkiye. *P. homoion* was previously reported 11 times from various freshwater fish species (Özer, 2021). Aydogdu et al.(2020 a) reported a prevalence of 11.7% in The European bitterling (*Rhodeus amarus*), which occurred in the same locality as this study and agrees with the prevalence of *P. homoion in* our study.

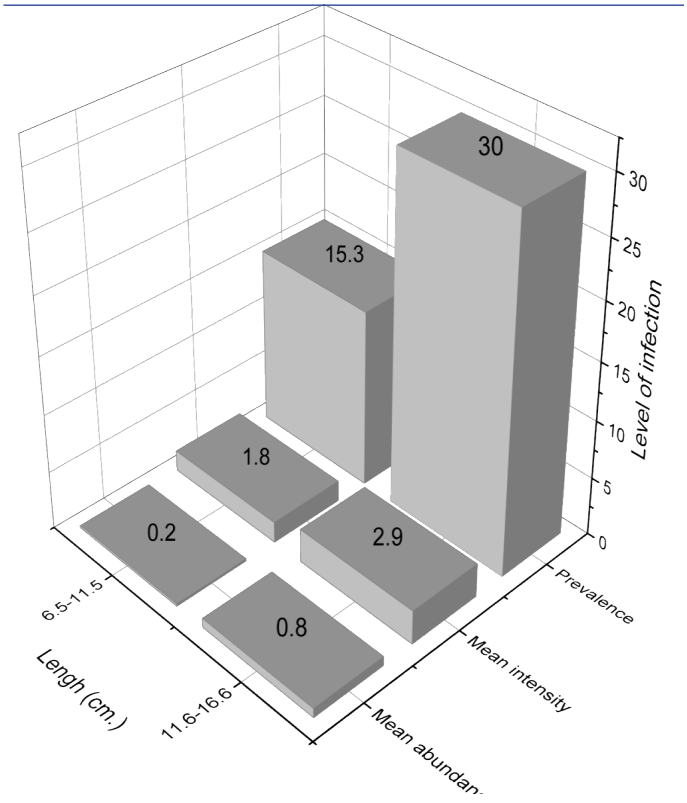

Figure 7. Distribution of the infection value of *Dactylogyrus vistulae* in *Squalius cii* from the Susurluk Basin, Balıkesir, according to the host length.

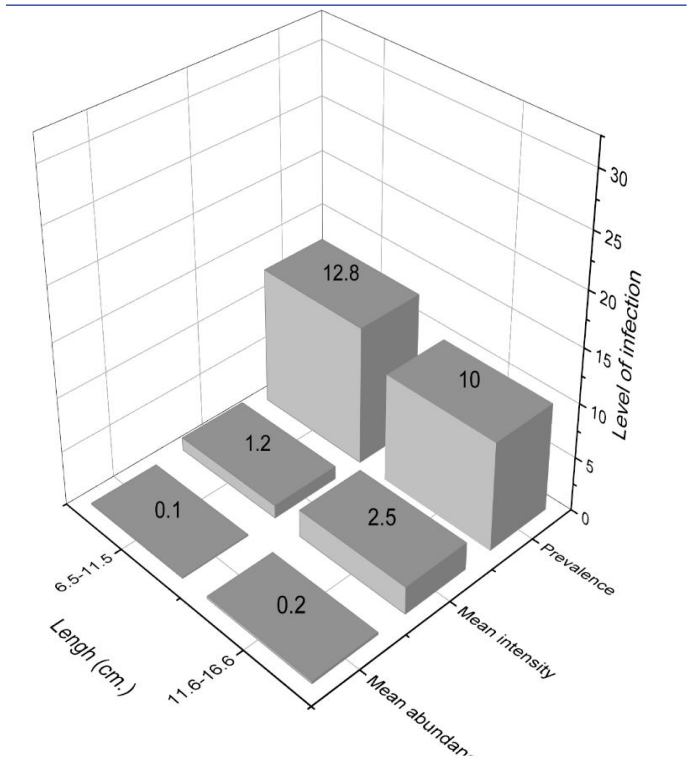

Figure 8. Distribution of the infection value of the *Paradiplozoon homoion* in *Squalius cii* from the Susurluk Basin, Balıkesir, according to the host length.

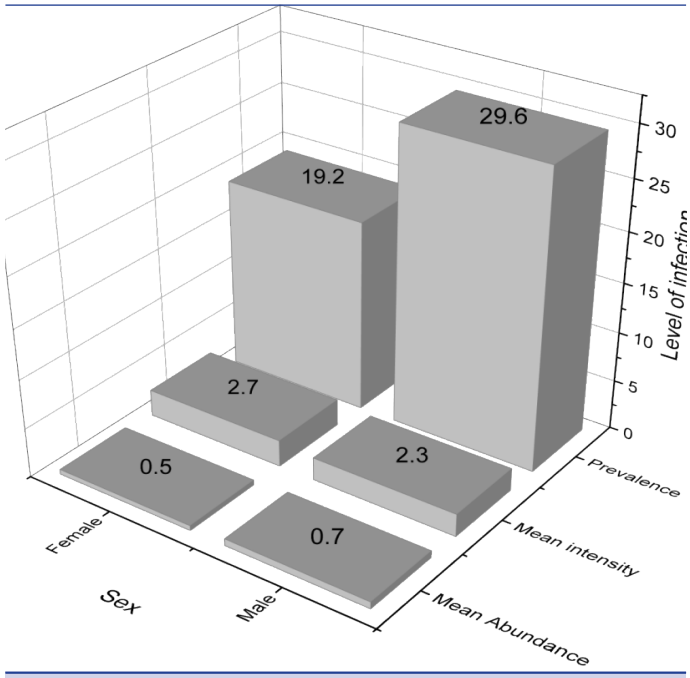

Figure 9. Presence of *Dactylogyrus vistulae* in *Squalius cii* in different sex groups.

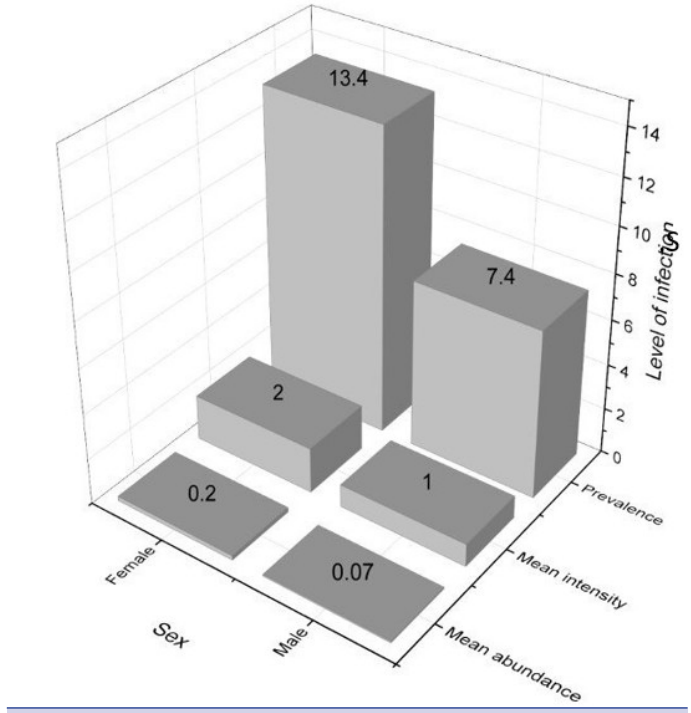

Figure 10. Presence of the *Paradiplozoon homoion* in *Squalius cii* in different sex groups.

With respect to the seasonality, the infection values of two ectoparasites helminths in this study varied, *D. vistulae*, the mean intensity was recorded the same in spring and summer while the prevalence reached high in spring. And this species was not detected in the autumn and winter samples. The finding in this study of *D. vistulae* was similar to that of Öztürk (2014); Aydogdu et al. (2015) and Elbay & Öztürk (2021) for S. *cephalus*, *S. anatolicus* and *S. recurvirostris*, respectively. In addition to these, *D. vistulae* has been identified by various authors many times in Türkiye from various freshwater fish species (Aydogdu et.al. 2001; Kurupınar & Öztürk 2009; Açıkel & Öztürk 2012).All of these authors noted the highest prevalence of *D. vistulae* infection in the spring season, similar to the value of our study, in the above-mentioned studies.

Regarding *P. homoion*, the highest prevalence of infection was recorded in winter. The parasite's infection intensity reached its peak in the summer season (3 parasite/ fish)(Fig. 6).

Many other researchers from Türkiye have investigated the seasonal variation of the infection rates of *P. homoion* in different fish species (*e.g.*Koyun2001; Öztürk 2005; Soylu 2007; Aydogdu et al.2020a,b).Aydogdu et al.(2020a,b) observed the highest infection prevalence value of *P. homoion* in the winter season from Manyas spirlin, *Alburnoides maynasensis*, from the Susurluk stream. Similarly, Soylu (2007) recorded the winter season to have the highest infection prevalence of this parasite species from flower fish, *Pseudophoxinus antalyae*. Contrary to these findings, Koyun (2001) did not encounter this species from the bleak *Alburnus alburnus* in the winter season. Similarly, Öztürk (2005) found *P. homoion* in all seasons (except winter) from Roach (*Rutilus rutilus*), and in the same study, the researcher only found this parasite in the summer season from Danube bleak (*Chalcalburnus chalcoides*). These authors pointed as due to different rates of parasite development in variations in temperature of different fish habitats.

The findings of the present study exhibit consistency with what recorded by Soylu (2007) and Aydogdu (2020a) and support this suggestion.

Generally, as the fish increased in size in this study for *D. vistulae*, the prevalence of infection values changed, the highest prevalence values of *D. vistulae* was recorded in the length classes II (11.6-16.6 cm), while the highest prevalence of *P. homoion* was observed in the length classes I (6.5-11.5 cm) et al.

In the present work, the highest prevalence and mean intensity values of *D. vistulae* recorded in the fish length 11.6-16.6 cm long (Fig. 7). The variation in the infection rates of *D.vistulae* and host fish length has been investigated by several researchers (Kurupınar & Öztürk2009; Açıkel & Öztürk 2012; Turgut et al. 2012; Öztürk2014; Aydogdu et al. 2015). Some of these authors (Kurupınar & Öztürk 2009; Öztürk 2014; Aydogdu et al 2015) found a positive relationship between the infection levels of *D.vistulae* and the length of the host fish. They found higher prevalence and mean intensity levels in medium and large fish. On the other hand, there are studies (Açıkel & Öztürk 2012) which found results the opposite, detecting infection prevalence and mean intensity values of *D. vistulae* the highest in young fish. Furthermore, Turgut et al. (2012) demonstrated no relationship between the mean intensity level of *D.vistulae* and host length. The present results agreed with Kurupınar & Öztürk (2009); Öztürk (2014) and Aydogdu et al.(2015) with a positive correlation between the abundance of parasites and the length of their host.

In case of *P homoion*, the prevalence of infection was highest in smaller fish 6.5-11.5 cm long, while the mean intensity was the highest in fish 11.6 – 16.6 cm long (Fig. 8).To the best of our knowledge, the relationships between *P. homoion i*nfection levels and host fish length in Türkiye have been studied by Koyun 2001; Soylu 2007; Öztürk, 2011 and Aydogdu et al. 2020a. Of these authors, only Aydogdu et al. (2020a) recorded its highest prevalence and mean intensity levels in large fish. In contrast to the findings of Aydogdu et.al.(2020a),others (Koyun2001; Soylu2007 and Öztürk 2011) found no host size-related effects on the infection of *P. homoion* in the increasing size classes of hosts.

As we know, in general, a positive relationship exists between the total number of monogenean parasite species per host and host length. This has been illustrated by several researches (e.g. Fisher & Kelso 1990; Bu and Song 1997; Aydogdu et al. 2003; Simkova et. al.2006; Açıkel & Öztürk, 2012) who found that the infection of *Dactylogyrus* increases with the length of the host fish. They also stated that in general, the number, prevalence and intensity of *Dactylogyrus* species increased with the length of the host fishes. They are of the opinion that larger sized host are probably present a larger gill surface for infection which the volume water that passes through the gills of larger sized fish which this conveys more oncomiracidium, larger sized fish had more surface are to accumulate parasites than smaller sized fish. Being a monogenean, one would expect *D.vistulae* to follow a general trend in the present study. The results of this study strongly confirmed the ideas mentioned above. Exactly as expected, *D. vistulae* preferred or accumulated on larger-sized fish with larger gill surface areas in the present study. Since larger-sized groups of *S. cii* were infested with *D.vistulae*, it could be concluded that larger sized host are large colonised surfaces area to parasites and more time to accumulate parasites than smaller-sized ones. To arrive at this conclusion, it should be noted that a sample size of 42 specimens per survey could possibly be sufficient to illustrate preferences for host size (Fig. 7).

In the light of the above information, a similar trend that infection of parasites increases with the length of the host fish is also expected from *P. homoion,* whereas the results of the present study indicated that this parasite had no preference for larger sized hosts. Therefore, we can conclude that *P. homoion* does not prefer or accumulate larger fish with larger gill surface areas in this study.

In the present study, the highest prevalence of infection of *D. vistulae* with respect to sex was highest in males, whereas it's the highest mean intensity level was observed in females. This agrees well with Özturk (2014) who found a higher prevalence of *D. vistulae* in male individuals of the chub. In the opposite of these findings, Elbay & Öztürk(2021)found that *D. vistulae* in male individuals of *Squalius recurvirostris* were heavily infected.

CONCLUSION

From the current study, two ectoparasitic helminth specimens were identified: the monogeneans, *Dactylogyrus vistulae*(Prost, 1967), and *Paradiplozoon homoion*(Bychowsky & Nagibina, 1959), were found on the gills. D. *vistulae* was the most common species in the host fishes and also found to be the maximum in number than *P. homoion*. In addition to these, other factors such as season, host length and sex were calculated to determine their effect on the prevalence, mean intensity and abundance of ectoparasitic helminth infection in the present study. In addition to these study findings, to the best of our knowledge, the two ectoparasitic helminth specimens recorded in this study were also recorded in studies in which different fish species were investigated in terms of ichtyohelminthological in Türkiye. However, since this study is the first ichtyohelminthological survey of *S. cii* in Türkiye, the host fish represents a new host record for two helminth specimens and thus, new knowledge has contributed to the geographical distribution and host range of these helminth species.

S. cii is a species consumed by local people in a narrow range with a small number of locations, but still has Least Concern (LC) status in the IUCN. While investigating the reasons for the decrease in this fish population in the future (massdeaths, overfishing), Monogenean group parasite epidemics, which are among the fish parasites and are especially lethal in aquaculture and rarely, in fish in the natural environment, should be evaluated and investigated as one of the causes of death. The necessity of investigating the species belonging to this group is because they have complex life history strategies adopted to ensure survival in unpredictable and opposing environments, such as strategies such as multiple reproductive mechanisms. With such studies, scientists will have the opportunity to evaluate the infection success of parasite species belonging to this group, depending on the host, environmental conditions and enemies, and their infection success depending on the seasons and host factors. Furthermore, data will be obtained about the complex life histories of the species belonging to this group and willcontribute to the determination of the causes of death that will occur in both cultured and natural fish populations in the future.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Committee Approval: The authors affirm that ethical approval is unnecessary for this study.

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Author Contributions: Nurten AYDOGDU and Hatice TORCU-KOÇ designed the study. Nurten AYDOGDU conducted field studies and Nurten AYDOGDU analysed the data. Nurten AYDOGDU and Hatice TORCU-KOÇ drafted this manuscript.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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