Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2024.132447 2025, 24(1) 123-139

Research Article

Endohelminth parasite communities of *Squalius cii*, including the zoonotic trematoda *Clinostomum complanatum*: Effect of host sex and size on community structure

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Keywords

Clinostomum complanatum,
Caryophyllaeides fennica,
Rhabdochona denudata,
Season,
Host size and sex

Article info

Received: July 2024
Accepted: September 2024
Published: January 2025

Abstract

An examination was carried out from spring 2020 (April) to winter 2021 (February) to determine the occurrence of endoparasitic helminth in European chub (*Squalius cii*) in the Susurluk basin from the Northwest region of Turkey. The infection levels of all identified endoparasitic helminths were also investigated in relation to host factors and seasons. In addition, the differences with their prevalence, mean intensity, and mean abundance were evaluated statistically. A total of 79 *S. cii* were examined, 33 fishes were found to be infected by one or more endoparasitic helminth specimens. Three species of endoparasitic helminth were identified on gill cavities and in the gastrointestinal tracts, respectively: *Clinostomum complanatum* (Rudolphi, 1814), *Caryophyllaeides fennica* (Schneider, 1902), and *Rhabdochona denudata* (Dujardin, 1845). *R. denudata* was the most common and most abundant species. The endohelminth infections were recorded in all seasons. *C. fennica* was recorded in spring and summer, while *C. complanatum* was encountered in spring and autumn, whereas it was not detected in summer and winter. The prevalences were higher in larger hosts based on body length in *C. complanatum*, *C. fennica*, and *R. denudata*, while a high mean intensity of *C. complanatum* and *R. denudate* was in smaller hosts. Male host fishes had higher prevalence levels of endoparasitic helminth infection. The present study provides the first record of the endoparasitic helminth of *S. cii* considered a valid species in Turkey and the first host record of three endohelminth species in *S.cii*.

Introduction

The freshwater ichthyofauna of Turkey consists of 427 species belonging to 20 orders, 37 families, and 97 genera (Çiçek *et al*., 2023). One of these genus, *Squalius* Bonaparte, 1837 is represented by 54 species worldwide (Van der Laan, 2014). Some of *Squalius* species were for long placed in the genus *Leuciscus*, Morphological and molecular studies, however, have shown that some species of this genus should be classified under the genus *Squalius* (*e.g*., Zardoya and Doadrio, 1999; Becer and Sarı, 2017). This genus belongs to the family Cyprinidae and shows broad geographical distribution in Europe and Asia (Özlüğ and Freyhof, 2011). In the inland waters of our country, 23 species of this genus have been recorded, and 9 of them are reported to be natural species (Çicek *et al*., 2023). One of the natural species, *Squalius cii* (Richardson, 1857) is distributed only in Anatolia, the northwestern part of Anatolia in Turkey and the island of Lesbos (Greece) (Stoumboudi *et al*. 2006; Öztürk and Küçük, 2017). According to our current literature information, only 106 of the 427 fish species distributed in Turkey's inland waters have been investigated ichthyo-helminthologically, and a total of 183 helminth parasite species have been reported (Özer, 2021). On the other hand, no study on the endoparasitic helminth communities of *S. cii* was found either in Turkey or in Greece. Moreover, according to our current literature knowledge, only one species belonging to the genus *Clinostomum* (Leidy, 1856) and *Caryophyllaeides* (Nybelin, 1922) have been recorded in Turkey, while four species (one unidentified species) belonging to the genus *Rhabdochona* Railliet, 1916 have been recorded so far (Özer, 2022). Therefore, this study aims to present the first data on the endoprasitic helminth fauna of this host fish in the inlandwaters of Turkey, to expand the number of identified species in the three genus and number of new hosts for the species belonging to the three genus in freshwater in Turkey, to determine how the infection prevalence, mean intensity and abundance values of endoparasitic helminth species vary according to seasons, size and sex of the host fish and to were analyzed by statistical method.

Materials and methods

European chub, *Squalius cii* (Richardson, 1857) were collected, using electrofishing, in one of branches of Simav stream in Susurluk basin in the northwest Anatolian region of Turkey (Fig. 1) from spring 2020 to winter 2021. Samples each comprising from 19-21 individuals of *S.cii*, representing fishes of small and medium-sized, were taken at attempting to collect equal number one in every three months. Seventy-nine individuals of *S. cii* were sampled. The fish were placed in plastic tanks containing aerated from the original locality and immediately transferred to the research laboratory for ichtyohelminthological investigation and examined for endoparasitic helminth approximately 2 to 3 hrs after capture. The fish specimens were killed by vertebral separation. Individual fishes were measured and were divided into two groups on body length. During dissection, on gill cavity and in the gastrointestinal tracts were examined under a Nikon binocular stereoscopic microscope for endo – helminth parasites.

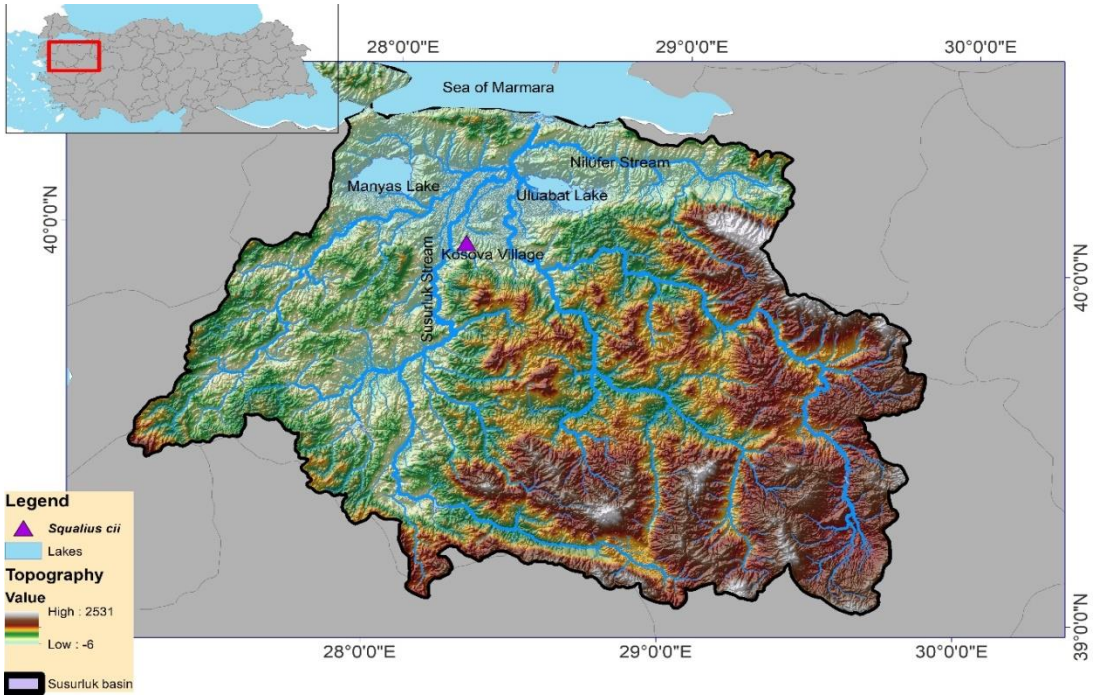

Figure 1: Sampling locality of *Squalius cii* in the Susurluk Basin Map.

In this context, the gastrointestinal tract of each fish was removed with dissection from the anus towards the head. The sex of every fish determined internally, once the abdominal cavity was opened. The intestines were removed and separated from the other visceral organs and placed in normal saline solution in petri dishes for examination using stereomicroscope. Furthermore, the eyes, liver, gallbladder, and swim bladder were also examined for endohelminth parasites or cysts. Specimens of endo – helminth parasites found in each dissected fish were counted, isolated from various organs of host fishes and then the freshly obtained parasites were washed (to ensure debris free) into saline solution contained in the petri-dish. The fixation of trematodes and cestodes was applied according to. Cribb and Bray (2010). Nematodes were in fixed in hot 4 % formalin, cleared and mounted in lactophenol without staining. Infection parameters for each species were recorded according to the seasons of collection, host length, and sex. Trematodes and cestode were identified according to the identification keys of Markevic (1951), Bychowkaya – Pavlovskaya (1962), Gussev *et al*. (1987), Gibson *et al*. (2002), Khalil *et al*. (1994), and Hoffman (1999). As for nematode species, the parasites were identified up to the species level according to the identification keys of Bychowkaya – Pavlovskaya (1962), Markevic (1951), and Moravec (1994). Ecological terms are as following; The prevalence was determined by dividing the number of infected fish × 100 by total number of fish examined; The mean intensity was calculated by dividing the total number of collected parasites by the number of infected fish, while abundance was evaluated by dividing the total number of collected parasites by number of (infected and uninfected) fish samples (Bush *et al*., 1997).

Photomicrographs were captured using a photographic camera mounted on an Olympus BX-50 research microscope. Kruskal-Wallis tests were applied to find significant differences in the mean intensity of the parasite species for seasons. The Mann-Whitney U test was used to determine the association between the intensity of endohelminth parasites for host length and sex. A significance level of $\alpha \leq 0.05$ was used. All statistics analyses were performed using SPSS v. 23.

Results

General infection

European chub, *Squalius cii* (Richardson, 1857) was examined seasonal basis for a period of four years from spring 2020 to winter 2021. The host fishes were researched to identify the endo-parasitic helminth fauna and their prevalence, mean intensity, and abundance. In addition, factors *e.g*. seasonal variation, sex and length of the host were also investigated to determine their effect on endo- parasitic infection of host fishes. A total of 79 *S. cii* were examined, of these 33 fishes were found to be infected by one or more endoparasitic helminth specimens including digenea, cestoda and nematoda.. In respect to the mixed infection, the present data reported that 41 % of examined host fishes had mixed species infection. During the study, a total of 65 endoparasitic helminth of 3 species, consisting of one of digenean, *Clinostomum complanatum* Rudolphi 1814, one cestode, *Caryophyllaeides fennica* (Schneider, 1902) and one nematode, *Rhabdochona denudata* (Dujardin, 1845) were found on gill cavity and in the intestines, respectively (Figs. 2 to 4).

Figure 2: *Clinostomum complanatum* metacercariae from *Squalius cii* a) single excysted on gills (scale bar=4µm) b) total view (scale bar=100µm).

Among these, the nematodes, *R. denudata* was the most numerically abundant species (Fig. 5). A total of 37 individuals were recorded from 18 fishes (prevalence 22.7%,

mean intensity 2.05 parasite/fish. *C. complanatum* was the second abundant nematode species with the prevalence, mean intensity and mean abudance of infection of 11.3 %, 2.4 parasite/fish and 0.2 respectively. In the study, the species *C. fennica* was found only in a very small number among the endoparasitic helminth of the host fishes. Figure 5 shows the infection values calculated for all parasite species.

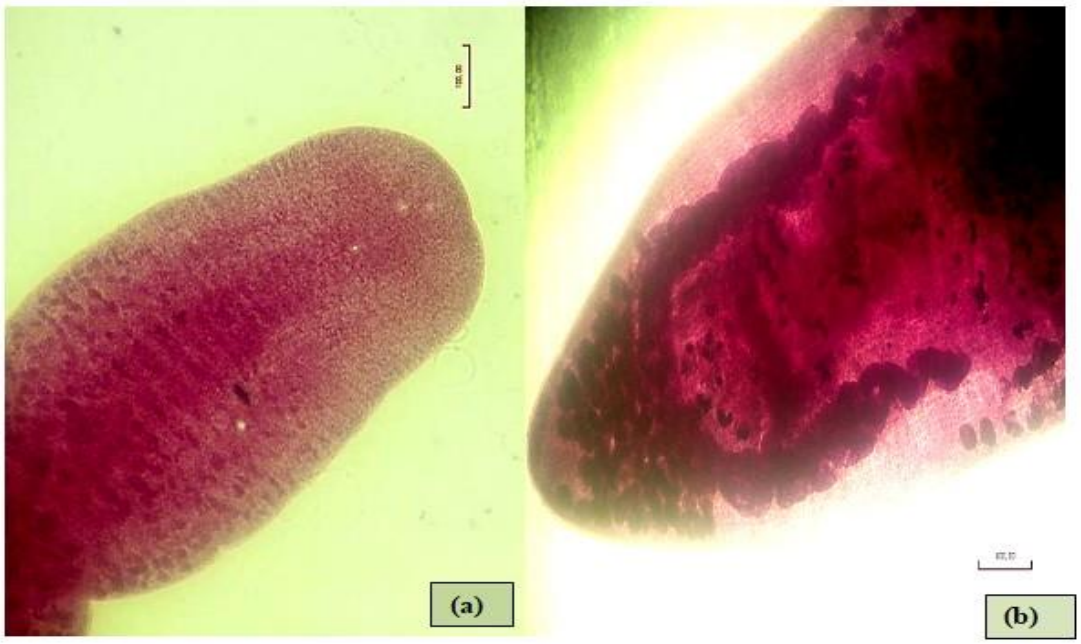

Figure 3: ***Caryophyllaeides fennica*** **a)anterior part (scale bar=100µm) b) posterior part (scale bar=100µm).**

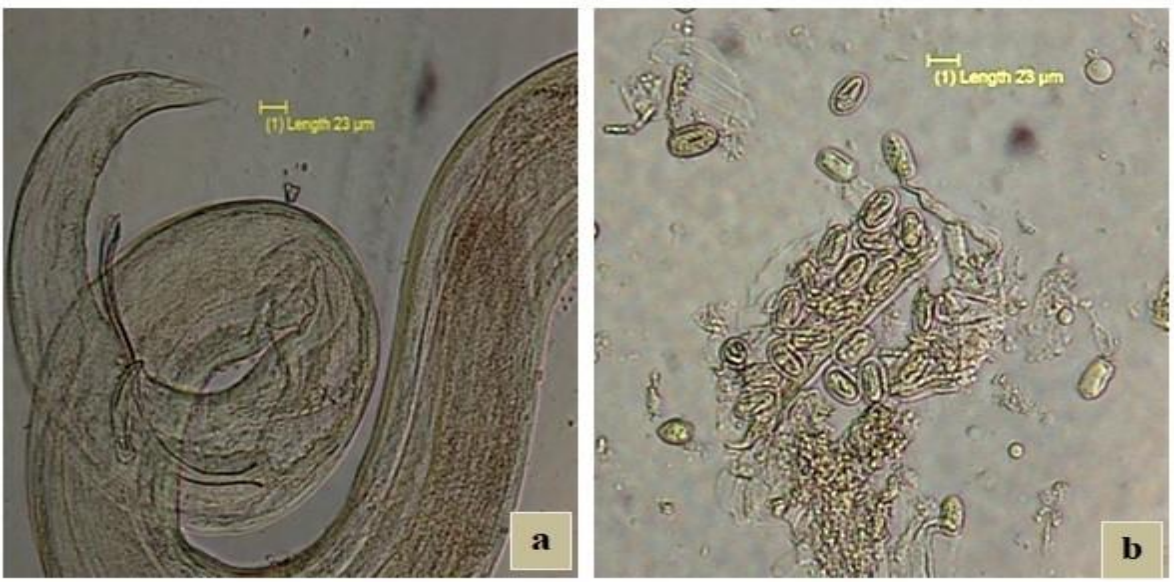

Figure 4: ***Rhabdochona denudata*** **from** ***Squalius cii*** **a) posterior end of male (scale bar=23µm) b)eggs (scale bar=23µm).**

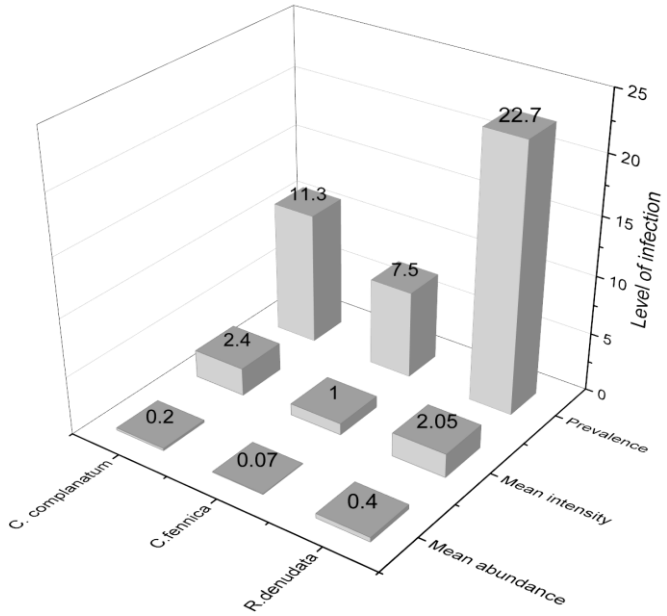

Figure 5: Distribution of infection value of endohelminth parasites in *Squalius cii* from Susurluk Basin, Balıkesir.

The distribution and infection values of helminth parasites according to season

The study was carried out seasonally from spring 2020 (April) to winter 2021 (February). The helminth infections were recorded during all seasons. *C. fennica* were recorded in spring and summer, while *C.complanatum* was encountered in spring and autumn, whereas it was not detected in summer and winter. No statistical difference was determined in the number of *C.complanatum* across seasons (p=0.05). Seasonal prevalence, mean intensity and mean abundance of parasites differed between seasons. *R.denudata* was present in all seasons, with the highest prevalence in summer (40%), and the lowest prevalence (10%), mean intensity (1 parasites/fish), and mean abundance (0.1) were observed in winter (Figs. 6 to 8). Despite this seasonal distribution of infection values, *R.denudata*, no statistical difference was found between the four seasons and the number of *R.denudata* in the host fish (p=0.139). The highest mean intensity for *C. fennica* was found to be similar in spring and summer, whereas the mean intensity values of *R. denudata* were observed to be the same in summer and autumn, The mean abundance of helminth parasites varied between 0,2 and 0,8 (Fig. 8) seasonally. In the current study, *C. fennica*, which was the least in number (n=6), was not found statistically significant in its seasonal distribution, as expected (p=0.41).

The distribution and infection values of helminth parasites according to the host fish sex

In the present study, 52 female individuals of *S. cii* were examined from which 15 were infected with the prevalence and mean intensity 65.8 % and 1.73 parasites/fish respectively while 27 specimens of male were dissected and 11 were infected among them.

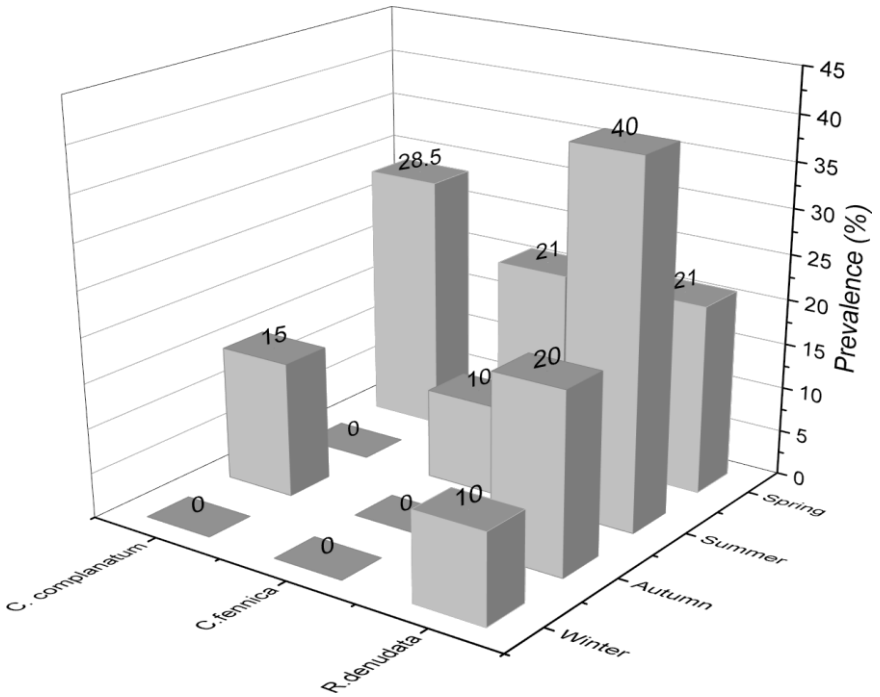

Figure 6: Prevalence value of endohelminth parasites in *Squalius cii* in different seasons.

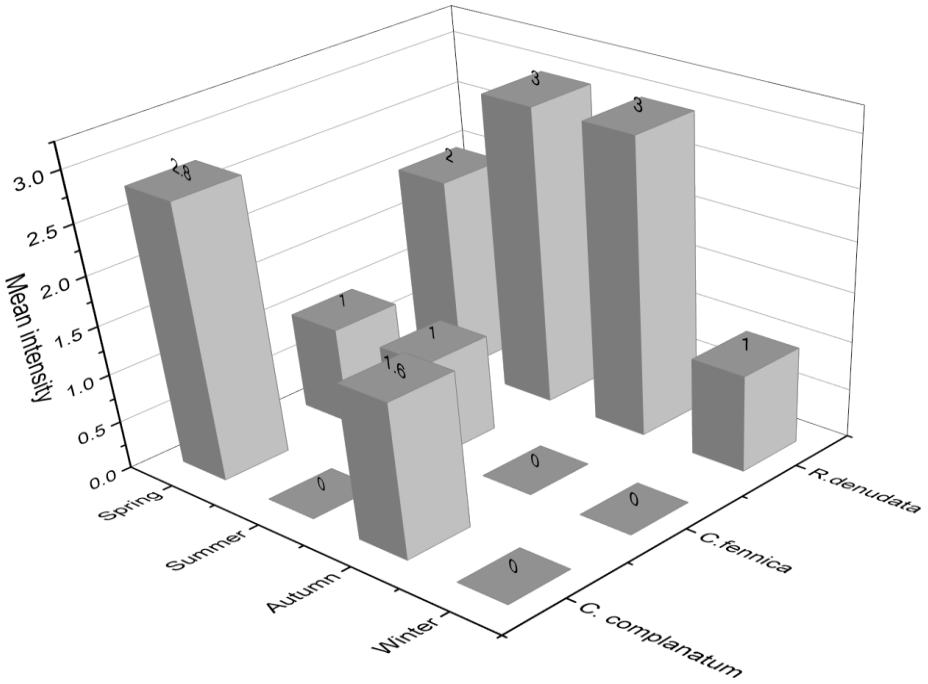

Figure 7: Mean intensity value of endohelminth parasites in *Squalius cii* in different seasons.

The prevalence and mean intensity of the endohelminthic infection in males were 34.13% and 1.44 respectively. Prevalence and mean intensity levels of *C. complanatum*, *C. fennica* and *R. denudata* were higher in males (Figs. 9 and 10). In addition, there was no statistically significant difference in the number of three parasite species and the sex of the fish.(p=0.14; p=0.39 and p=0.23, respectively).

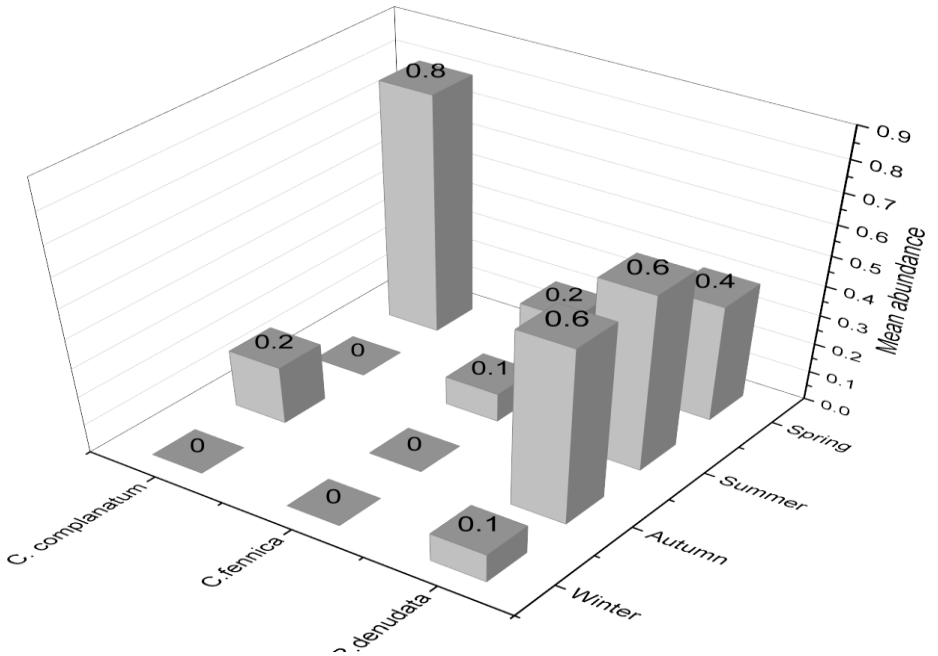

Figure 8: Mean abundance value of endohelminth parasites in *Squalius cii* in different seasons.

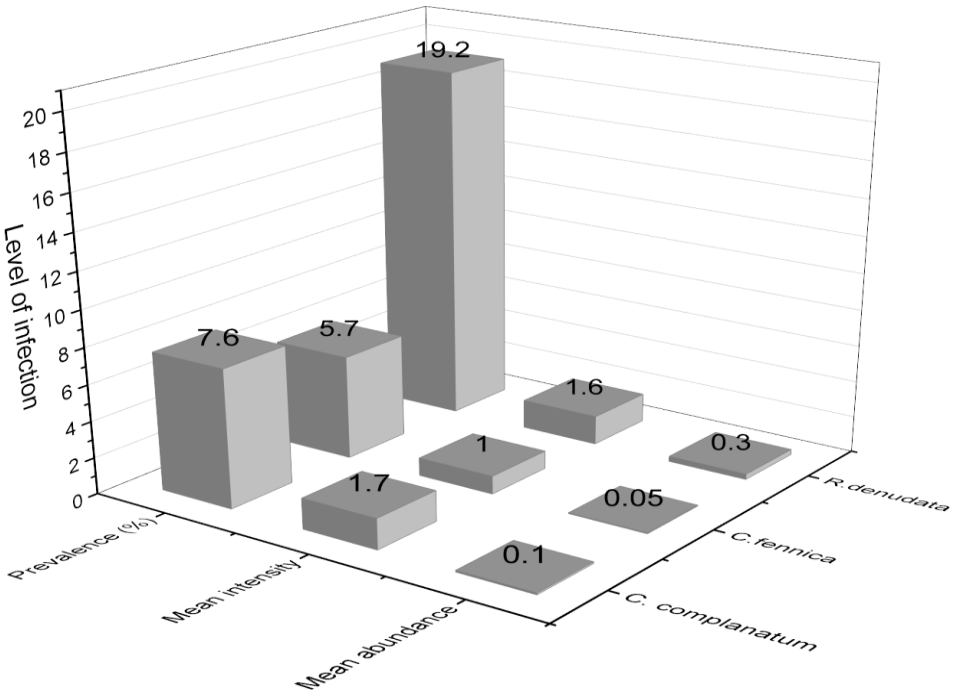

Figure 9: Presence of endohelminth parasites in female *Squalius cii.*

The distribution and infection values of helminth parasites according to the host length classes

Host fish specimens were divided into two groups according to their sizes. The highest prevalence of *C. complanatum*, *C. fennica* and *R. denudata* were recorded in length classes II (11.6-16.6 cm). The mean intensity of endohelminth parasites also showed differences in two length classes. The mean intensity levels of *C. complanatum* and *R. denudata* reached their maximum in the smaller, whereas in *C. fennica*, the mean intensity is equal in both length classes.

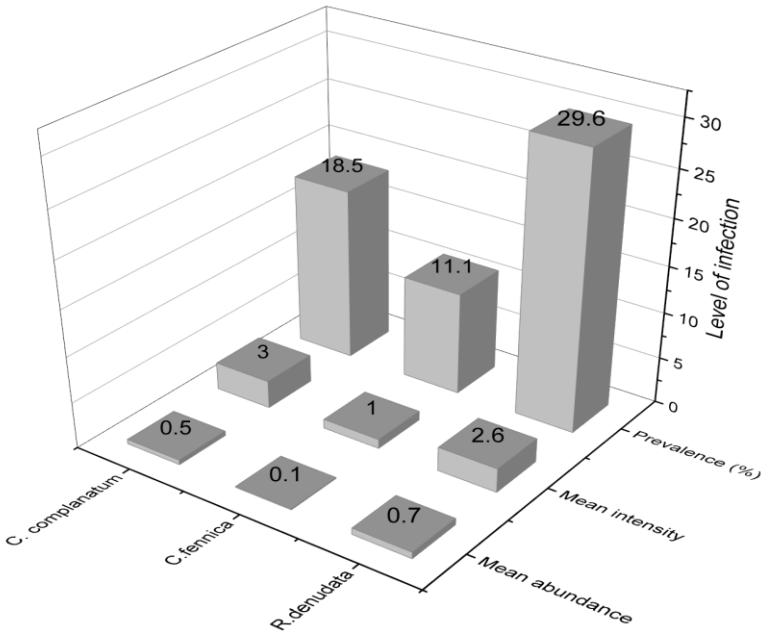

Figure 10: Presence of endohelminth parasites in male *Squalius cii.*

In addition, the mean abundance of helminth parasite species was almost equal in length classes (Fig. 11; Table 1). There was no statistically significant difference in the number of *C. complanatum* and *C. fennica* between host length classes (*p*=0.618 and 0.347), whereas there was correlation between the length of *S. cii* and the numbers of *R. denudata* (*p*=0.031).

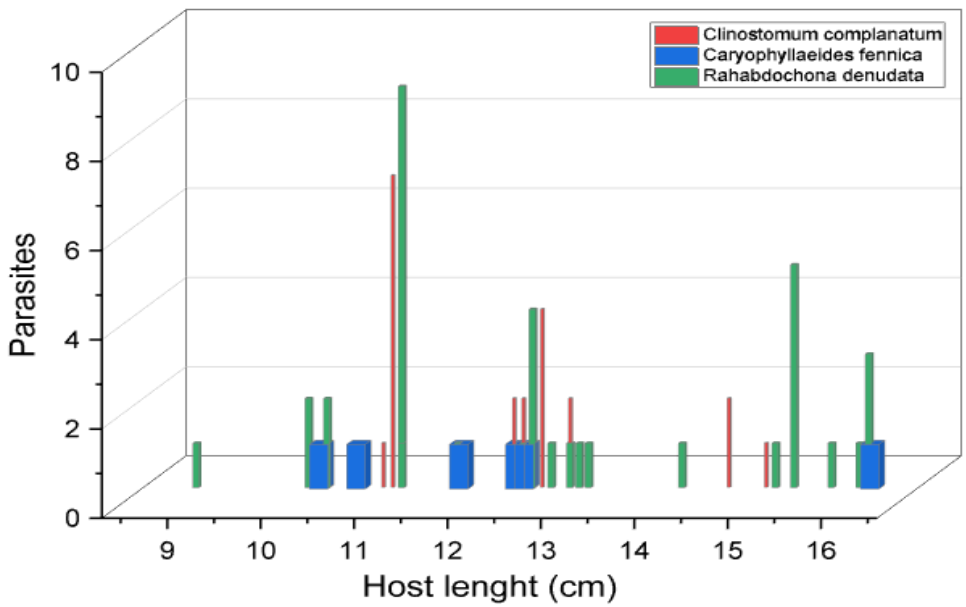

Figure 11: Presence of endohelminth parasites in length *Squalius cii.*

Table 1: Distribution of infection value of helminth parasites in *Squalius cii* from Susurluk Basin, Balıkesir according to the host length.

Fish Length Groups (cm)	Parasite species	Infected Fish	Prevalence (%)	Mean intensity	Abundance	Total parasite no
6.5-11.5 (n=39)	*C. complanatum*	2	5.1	4	0.2	8
	C. fennica	2	5.1	1	0.05	2
	R. denudata	5	12.8	2.8	0.3	14
11.6-16.6 (n=40)	*C. complanatum*	7	17.5	2	0.3	14
	C. fennica	4	10	1	0.1	4
	R. denudata	13	32.5	1.7	0.5	23

Discussion

This study reports new host records and new localities were added for the distribution of three endohelminth specimens. The dominant parasite species in the helminth assemblage *Rhabdochona denudata* (Dujardin, 1845) had the highest prevalence, intensity, and abundance, whereas *Caryophyllaeides fennica* (Schneider, 1902) had the lowest prevalence, intensity, and abundance.

In the present study, nine specimens were infected, *Clinostomum complanatum* Rudolphi, 1814, (prevalence 11.3%, mean intensity 2.4). It was previously recorded in Turkey from *R. amarus*, *Cyprinus carpio*, *Alburnus* sp., *Chondrostoma* sp., *Varicorhinus* sp., *Luciobarbus escherichi*, *Capoeta tinca*, *Scardininus erythrophthalmus*, *Sander lucioperca*, *Lepomis gibbosus*, *Perca fluviatilis*, *Rutilus rutilus*, and *Squalius cephalus* (Burgu *et al*. 1988; Öge and Sarımehmetoglu 1996; Davidova *et al*., 2011; Çolak 2013; Soylu, 2013, 2014; Simsek *et al*., 2018; Aydogdu *et al*., 2020). As to the infection results of this species in Turkey: the infection prevalence value was 15.4% in *P. fluviatilis* from Lake Sıgırcı (Soylu, 2013); 53.8%, 17.2%, 7.7 in *P. fluviatilis*, *S. erythrophthalmus*, *R. rutilus* respectively from Lake Gala; 13.1% in *P. fluviatilis*, 8.3% in *Sander lucioperca* and *Lepomis gibbosus* from Lake Sıgırcı (Çolak, 2013); 13.7 in *R. amarus* from Susurluk stream (Aydogdu *et al*., 2020). Comparing these values to the above-mentioned, the prevalence agrees with that reported by Aydogdu *et al*. (2020) who reported 11.7%, prevalence in hosts in the same habitats are explained by similarities of biotic and abiotic factors in locality, differences are identified with diversity of biotic and abiotic factors varying from one aquatic ecosystem to the other (Williams and Jones, 1994; Galli *et al*., 2001; Mouritsen and Poulin, 2002; Açıkel and Öztürk, 2012; Koyun *et al*., 2015). Because different geographic regions with different limmologic properties show very little homogeneity (Öztürk, 2017).

Caryophyllaeides fennica (Schneider, 1902) is a rare parasite of *S.cii* in the region compared to other species,6 of 79 the host fish (7.5%) were infected with 6 parasites. This species has been recorded twice in Turkey from rudd (S. *erythrophthalmus)* (Öztürk and Altunel, 2001) and *S. recurvirostris* (Elbay, 2014). They reported that in 10 out of 135 (7.4%) of the rudd (*S. erythrophthalmus*) from Lake Manyas, 14 *C. fennica* were collected, while in

Düzağaç-Akdeğirmen Dam Lake 16 out of 66 (24.2%) *S. recurvirostris* (Elbay 2014) were infected.

The findings in this study of *R. denudata* infection values in *S.cii* have recorded prevalence of 22.7%, mean intensity of 2 and mean abundance 0.4 respectively. Moravec (1994), reported that this species parasitizes in *Leuciscus, Rutilus, Scardinius* and *Alburnus* heavily. *R.denudata* occurs in various fishes in Turkey, Only *Leuciscus cephalus*, and *Alburnus alburnus* species corresponds with the list provided by Moravec (1994) (Aydogdu *et al*., 2001; Aydogdu and Selver, 2006; Öztürk 2014). Prevalence vary from 27.7 % in *Leuciscus cephalus* from Dogancı Dam Lake (Aydogdu *et al*., 2001), 41.6% in *Alburnus alburnus* from Mustafakemalpaşa Stream (Aydogdu and Selver, 2006) and 60.8% in *S. cephalus* from Akçay Stream (Öztürk, 2014). In the present study prevalence, it was 23.4 % similar to that reported by Aydogdu *et al*. (2001). Diversity of common parasitic species in different fish species occurring in the same or nearby habitats and their infection rates are similar since these localities have similar biotic and abiotic factors (Açıkel and Öztürk, 2012; Öztürk, 2014; Koyun *et al*., 2015; Öztürk, 2017).

A definite seasonal effect was noted for all three endohelminth species and prevalence and mean intensity of infection varies from one species to species throughout the entire research period. *R. denudata* varied between 10 and 40 % highest in summer lowest in winter. However, the mean intensity values change very little between the seasons. This observation was similar to that of Öztürk (2014) who observed the highest prevalence for *R. denudata* in chub (*Squalius cephalus*) in summer. Similar findings have been also reported by Aydogdu *et al*. (2001) in the cub (*Leuciscus cephalus*) in Doğancı Dam Lake by this parasite species. In this study seasonal variation per species, a total 47 specimens of *R. denudata* were recovered from 21 of 77 individuals of *L. cephalus* examined. This species was detected in the months of May, June, September, and November, while no parasites were found in the periods of December - February, July - August. The highest intensity in this species was recorded in June (9 specimens).

In the case of *C. complanatum*, in this study, the metacercariae were recorded only in spring and autumn. The prevalence was highest in spring (28.5%). The highest mean intensity and mean abundance were also found in spring (2.8 and 0.8, respectively). Seasonal variation in infection was reported in only one (Soylu, 2013) former study listed only one (Burgu *et al*. 1988; Öge and Sarımehmetoglu 1996; Davidova *et al*., 2011; Çolak 2013; Şimşek *et al*., 2018; Aydogdu *et al*. 2020). In contrast to our finding, Soylu (2013) reported the same intensity in perch (*Perca fluviatilis*) in Lake Sığırcı.

In terms of seasonality, the other endoparasitic helminth species, *C. fennica* was recorded rarely and the total was 6 specimens (4 specimens in spring and 2 specimens in summer). Öztürk and Altunel (2001) found *C. fennica* in *S. erythrophthalmus* from Lake Manyas. In addition, these authors found this parasite in summer, autumn, and winter samples and determined that the infection rate was

66,6% in June and the number of parasites encountered in a fish varied between 1-3. However, they reported no parasite infection during winter and spring. In contrast, Elbay (2014) noted the prevalence of this parasite species was high in *S. recurvirostris* in winter (54, 5).

The changes in seasonal prevalence and mean intensity values in parasite infection in freshwater fish mainly depend on parasite species, host species and environmental factors of the local habitats. The occurrence of parasites is influenced by their maturation, life cycles, and host specificity (Bari *et al*., 2015; Koyun *et al*., 2015). Factors determining the host fish species are their feeding habits, the choice, composition of food, hormonal status, immunological response, migration, etc (Aydogdu *et al*., 2001; Kurupınar and Öztürk, 2009; Öztürk, 2014; Bari *et al*., 2015; Koyun *et al*., 2015). Environmental factors are as follows: the presence or absence of an infective intermediate host, the seasonal abundance of the intermediate host, temperature, etc (Koyun *et al*., 2015). Seasonal temperature changes in habitats where fish species live affect both the feeding activities of fish species and the seasonal abundance of intermediate hosts of endoparasitic helminth species. As the water temperature decreases, especially in autumn and winter, both the feeding activities of the fish and the seasonal abundance of the intermediate hosts of endoparasitic helminth species decrease (Barson, 2004). Thus, the rate of contamination of endoparasitic helminth species to the definite fish host is reduced (Simkova *et al*., 2005; Burgmer *et al*., 2007; etc.). Differences in seasonal variability in infection may be due to a combination of factors in parasite species, the host, and environmental conditions. However, it is difficult to distinguish which factors influence as they are interrelated.

Generally, as the fish increased in size in this study, the prevalence of infection values changed, the highest prevalence values of *C. complanatum*, *C. fennica*, and *R. denudata* were recorded in the length classes II (11.6-16.6 cm) based on body length.

There were also differences between the fish length and infection values according to three endoparasitic helminths in this study. The highest prevalence value and total number of *C. complanatum*, *C. fennica*, and *R. denudata* were recorded in length classes II 11.6-16.6 cm, while their intensities reached their maximum values in fish between 6.5-11.5 cm. According to Öztürk and Altunel (2001) studies on Caryophyllaeid parasite species have shown that the infection of *C. fennica* may vary in relation to length of the host fish. And they recorded this parasite species infection in length classes 13 -18 cm. Similar results by Moravec and Scholz (1991), Elbay (2014), and Açıkel and Öztürk (2016) indicated that some cestode species parasitising in chubs, the number increased with the length of host fishes. They were postulated in the special diet of the different length host fish specimens preferring potential parasite intermediate host seems to be the main reasons for changes of infection values of cestode species between fish length classes. Furthermore, Kennedy (1969) indicated that the infection status of the endoparasitic helminth species in the host fish specimens

varies according to whether the intermediate hosts are present in the environment or whether they prefer these intermediate hosts as food depending on the length of the host fish. The above-mentioned authors' results and opinions are complementary quality each other and support why the number and infection values of *C. fennica* are higher in length groups 11.6-16.6 cm in the present study. As for *R. denudata*, the findings in this study of *R. denudata* at the highest levels of prevalence in the host fishes were recorded in length classes II (11.6-16.6 cm) (33.3%). The study by Kestek (2018) reported that the prevalence of *R. denudata* was high in Antalya minnow in length classes 12.9 - 16.8 cm (14.4%), while Koyun (2021) recorded the highest prevalence in Mesopotamianspiny eel in length classes 200- 300 mm (33%). The findings of our study are similar to those of Kestek (2018).

In the present study, the prevalence and mean intensity of infection of C. *complanatum, C. fennica*, and *R. denudata* with respect to sex were highest in male. In this context, as for *R. denudata,* Öztürk (2014) found that this species in male individuals of chub was heavily infected. In the opposite of these findings, Koyun (2021) found the maximum parasite number of *R.denudata* in female individuals of Mesopotamian spiny eel. Alternatively, a study by Kestek (2018) found higher levels of infection of this species in female hosts. However, the author could not record a statistical significance between the infection values of this species and the host fish sex. The higher prevalence of *R. denudata* in male hosts in this study is consistent with Öztürk (2014).

Conclusions

As a result of the conducted study of 79 specimens *S.cii* caught from the Susurluk basin from the Northwest region of Turkey, 3 parasite species were identified:, one of digenean, *Clinostomum complanatum* Rudolphi, 1814, one cestode, *Caryophyllaeides fennica* (Schneider, 1902) and one nematode, *Rhabdochona denudata* (Dujardin, 1845) were found on gill cavities and in the intestines respectively. *R. denudata* was the most common species in the host fishes and also was found to be more in number than the rest two parasite species. In addition to these study findings, to our knowledge, the host fish represents a new host record for all endohelminth species and thus, new knowledge has been contributed to the geographical distribution and host range of these helminth species

Acknowledgments

We would like to thank Professor Annemarie Oldewage of Rand Afrikaans University and Professor Lesley Warner of the Australian Museum for their important contributions, for reading the manuscript and for providing valuable comments.

Conflicts of interest

All authors have read and agreed to the published version of the manuscript declare that they have no conflicts of interest. The authors also have nothing to disclose.

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