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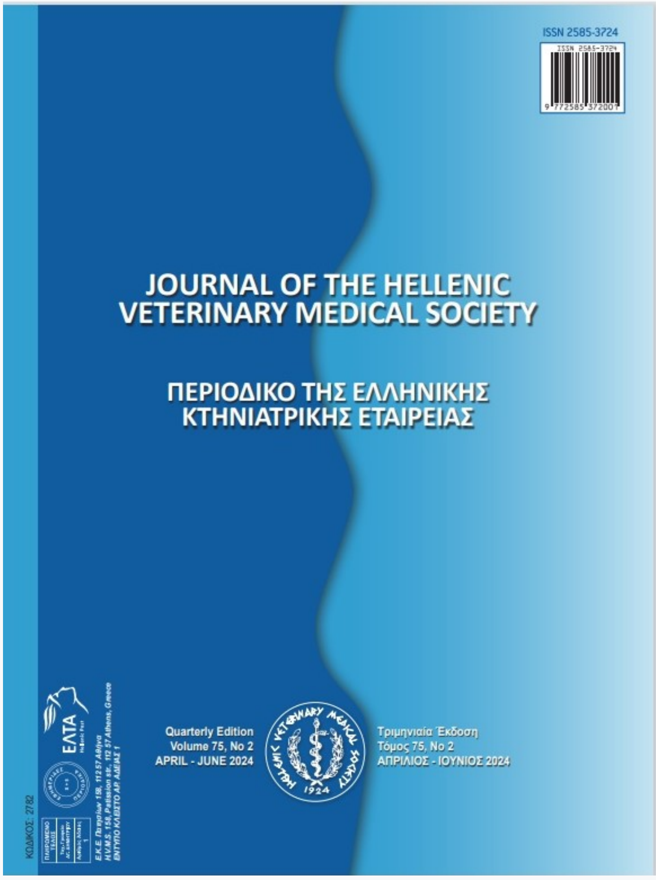

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N Aydogdu, N Emre, R Aco-Alburqueque, S Mattıuccı, A Aydogdu

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Observations on Helminth Parasites in Endemic and Native Fish from Susurluk Basin, Türkiye Using a Genetic- Molecular Approach: With New Host and Geographical Records

N. Aydogdu[1*], N. Emre[2], R. Aco-Alburqueque[3], S. Mattıuccı[3], A. Aydogdu[4]

[1]Balıkesir University, Graduate School of Natural and Applied Sciences, Department of Hydrobiology, Balıkesir, Turkiye

[2]Akdeniz University Faculty of Education, Department of Mathematics and Science Education, Dumlupınar Boulevard,Campus 07058/Antalya/ Türkiye

[3]Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, P.le Aldo Moro, 5, 00185 Roma, Italy

[4] Bursa Uludag Universty, Faculty of Veterinary Medicine, Department of Aquatic Animal Diseases, Görükle- Bursa, Turkiye

ABSTRACT: In this study, a total of 50 individuals of three endemic fish species, namely *Chondrostoma angorense* (n=13), *Oxynoemacheilus simavicus* (n= 9) and *Oxynoemacheilus angorae* (n= 12), and one native fish species, *Silurus glanis* (n=16) were examined for helminth parasites between February 2021 and December -2021 from Nilüfer stream and Simav stream, in the northwest Anatolian region of Turkey. A total of 5881 parasite species of five helminths were found in four of the fish species consisting of 2 monogenean: *Paradiplozoon homoion* (in *C. angorense*) and *Ancylodiscoides vistulensis* (in *S. glanis*), one digenean: *Clinostomum complanatum* metacercariae (in *C.angorense*), 2 nematodes: *Contracaecum rudolphii* B (in *C.angorense*, *O. simavicus* and *O. angorae*) and *Eustrongylides excicus* larvae (in *S. glanis*). *A. vistulensis* was found to be the highest number compared the remaining parasite species. The present work reported for the first time the occurrence of P. homoion, C. complanatum metacercariae and C. rudolphii B in C.angorense, and C. rudolphii B in O. simavicus and O. angorae. This depicted new host records and geographical distribution for those five helminth parasites.

Keywords: Susurluk basin; endemic and native fish; helminth parasites

Corresponding Author:
Aydogdu N, Balıkesir University, Graduate School of Natural and Applied Sciences, Department of Hydrobiology, Balıkesir, Turkiye
E- mail: dactylogyrus@hotmail.com

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INTRODUCTION

According to recent findings, a total of 409 freshwater fish species are recognized within the political boundaries of Turkey, and 194 of these fish species are endemic to Turkey (Çiçek et al., 2018). As far as we know, there are no studies or it is known that there are limited numbers of studies on the ichthyoparasitological investigation of various freshwater fish species still widespread in our country today. To increase the number of studies in this field, scientific studies investigating fish species in terms of ichthyoparasitology started in the 1960s and gained momentum in the 2000s. Despite the increase in the number of studies in this field in recent years, according to the latest findings (Özer, 2021), the number of parasite species reported from freshwater fishes is only 186. This number is quite low when compared to the number of species recorded in studies conducted in this area in neighboring countries of Turkey. Moreover, the number of studies investigating endemic fish species, represented by 194 species distributed in inland waters of Turkey, in terms of ichthyoparasitological is also very low. Exactly confirming the above information, to the authors' knowledge and available references, there are no records of ichthyohelmintological studies for *O. simavicus* and only one ichthyohelmintological study is available on *C. angorense* (Innal et al.,, 2020).

The present study aimed to (i) characterize the community of helminthic parasites from endemic and native fish species collected from two localities of Susurluk basin, Turkey using a genetic-molecular approach (ii) improve the epidemiological data, geographical distribution, and host range of helminthic parasite in Turkish waters.

MATERIALS AND METHODS

Sampling and parasitological analysis

A total of 50 individuals from three endemic *Chondrostoma angorense*, *Oxynoemacheilus simavicus*, *Oxynoemacheilus angorae* (n= 12), and one native, *Silurus glanis* fish species were collected for the presence of helminth parasites. The fish were collected by electrofishing and transported to the laboratory in ventilated plastic tanks from the following localities in Susurluk basin, Turkey. *Chondrostoma angorense* (n=13); *Silurus glanis* (n=16) Susurluk stream, (39^0 48 ' 973"K, 28^0 10' 714"D) and *Oxynoemacheilus simavicus* (n= 9); *Oxynoemacheilus angorae* (n= 12) Nilüfer stream (40°10'44.8"N 28°58'13.0"E), Bursa. The sampling localities of fish species are shown in Figure 1.

The fish were measured (total body length, TL, 5 mm accuracy) and weighed (total body weight, TW, in g) before inspection for parasites. For the collection of parasites, the external body, oral cavity, gills, branchial cavity, body cavity, stomach, small intestine, large intestine, pyloric caeca, mesenteries, gonads, heart, branchial artery, swim bladder, kidneys, liver, spleen of the fish were carefully examined using a stereomicroscope (SMZ 745 Nikon and SZ61 Olympus). Monogenea specimens were removed and were made permanent with a mixture of glycerine-ammonium picrate (Malmberg et al., 1957; Bylund et al.,1980). Trematodes were fixed in 4 % formalin solution and stained with acetocarmine stain, rinsed dehydrated, cleared in Xylene, and mounted in Canada balsam. Nematodes were fixed in hot 4 % formalin, cleared, and mounted in lactophenol without staining. The identifications of all specimens were performed according to the diagnostic morphological keys of Gussev (Gussev,1985, 1987;Bychovskaya - Pavlovskaya, 1962 ; Markevic, 1951; Yamaguti,1985a,b,c; Khotenovsky,1985; Moravec, 1994; Dawes,1968; Hoffman 1999; Gibson et al.,2002; Khalil et al.,1994). and available references using an optical microscope. The levels of prevalence, mean intensity, and mean abundance of infection as defined by Bush et al. (1997) were calculated. Standard statistical computation (standart deviation) was carried out using Microsoft Excel (Office 2000). Finally, the collected nematode larvae were washed in saline solution, fixed in 70% ethanol, and delivered to the Laboratory of Parasitology, Department of Public Health and Infection Diseases of "Sapienza-University in Rome", for molecular identification. The photomicrographs of all parasite specimens were taken using a photographic

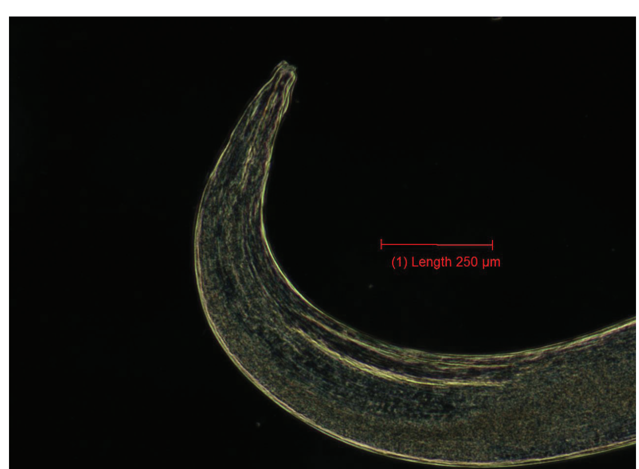

Figure 1. *Contracaecum rudolphii*-B anterior

camera-mounted Leica DMR microscope with phase contrast and BX-50 Olympus with a research microscope.

Molecular Analyses

The samples fixed in alcohol were first washed in PBS and distilled water before the DNA extraction. The total DNA of each single larvae was extracted using the Quick-gDNA™ Miniprep Kit (ZYMO RESEARCH) following the standard manufacturer recommended protocol. The mitochondrial cytochrome C oxidase subunit II (cox2) gene was amplified using the primers 211F (5-TTTTCTAGTTATATAGATTGRTTYAT-3) and 210R (5- CACCAACTCTTAAAATTATC-3) (Nadler and Hudspeth 2000). Polymerase chain reaction (PCR) was carried out according to the previously described procedures Mattiucci et al. (2008). PCR amplicons were sent to an external laboratory for sequencing (Bio-Fab Research, Rome, Italy). Contiguous sequences were assembled and edited using MEGA X v. 11. (Kumar, 2018). Sequence identity was checked using the Nucleotide Basic Local Alignment Search Tool (BLASTn) (Morgulis et al., 2008).

Additionally, to confirm the morphological identification of diplozoid species, the molecular analysis was performed as previously described by Aydogdu et al. (2020 a , 2020 b). And Sequence data was published to GenkBank (Genbank accession number: OP559061)

RESULTS

Of the 50 fishes, representing 3 endemic and one native species were examined. Thirty-seven fish individuals were found to be infected with one or more parasite species. A total of 5881 parasites of 5 species were identified in the fishes consisting of 2 monogeneans: *Paradiplozoon homoion* (in *C. angorense*) and *Ancylodiscoides vistulensis* (in *S. glanis*), one digenean: *C. complanatum* metacercariae (in *C.angorense*), 2 nematodes: *Contracaecum rudolphii* B (in *C.angorae*, *O. angorae* and *O. simavicus*) and *Eustrongylides excicus* larvae (in *S. glanis*). The prevalence, mean intensity, abundance, range, and total parasite number of these parasites are shown in Table 1.

The Ankara nase, *Chondrostoma angorense*, averaged 70.2g (± 53.8g, range 36.1-215.8g) in weight and averaged 20.4 cm (± 3.5cm, range 17.1-28.6 cm) in total length. Among the 13 examined *C. Angorense*, all individuals were found to be infected with one or more parasite species. The most prevalent and abundant parasite species were *C. Rudolphii* B (Figure 1- 2) in this fish. All fish studied were

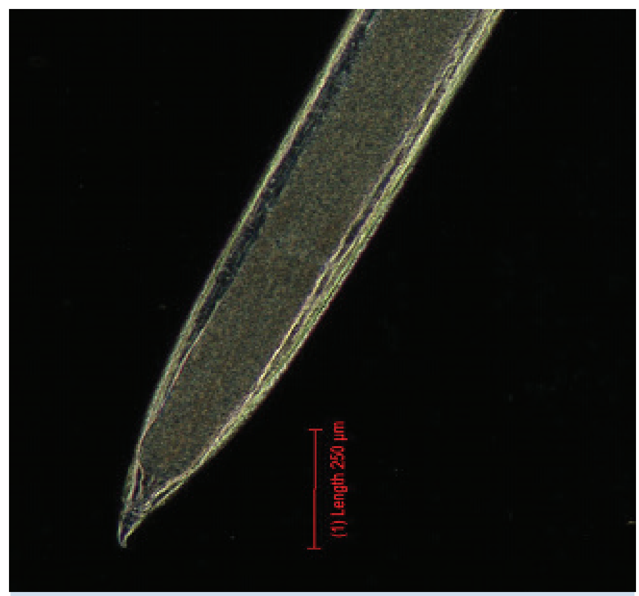

Figure 2. *Contracaecum rudolphii*-B posterior

Table 1. Distrubition of infection value of helminth parasites in endemic and native fish from Susurluk basin, Turkey

Host and parasite species	Prevalence (%)	Mean intensity±SD	Mean abundance	Range	Total Parasite no
Chondrostoma angoranse (n=13)					
Paradiplozoon homoion	61.5	4.2±4.4	2.6	1-11	34
Clinostomum complanatum metacercariae	53.8	1.5±0.5	0.8	1-2	11
Contracaecum rudolphii-B	100	10.9±8.3	10.9	3-28	142
Oxynoemacheilus simavicus (n= 9)					
Contracaecum rudolphii-B	33.3	2±1.7	0.3	1-4	6
Oxynoemacheilus angorae (n= 12)					
Contracaecum rudolphii-B	41,6	1,6±0.8	0,6	1-3	8
Silurus glanis (n=16)					
Ancylodiscoides vistulensis	100	355,8±280.6	355,8	22-1214	5694
Eustrogylides excicus larvae	56,2	5,1±4.1	2,8	1-15	46

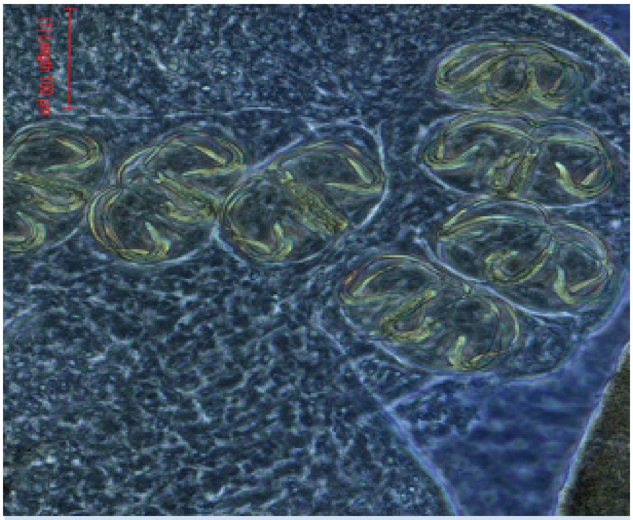

Figure 3. *Paradiplozoon homoion* haptor and clamps

Figure 4. *Clinostomum complanatum* metacercariae total view

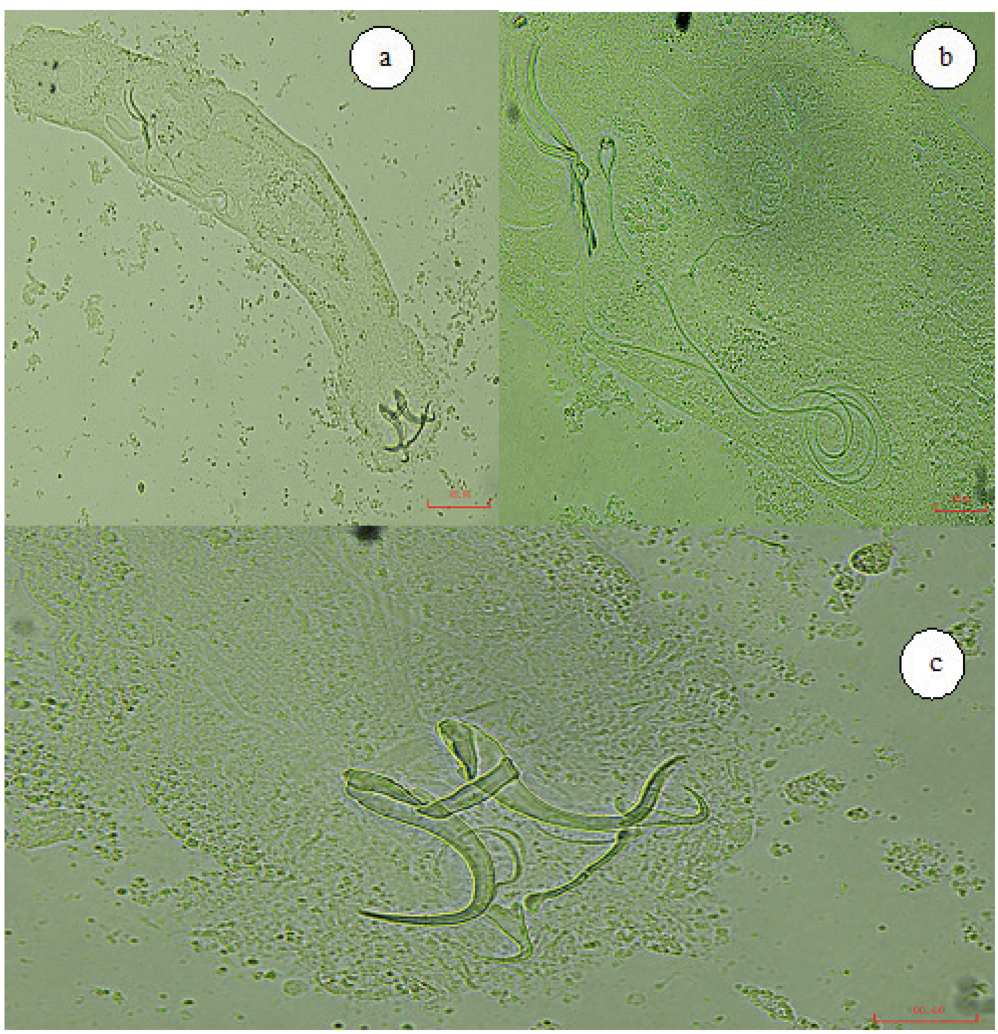

Figure 5. *Ancylodiscoides vistulensis* a) Total view b) copulatoion organ c) haptor

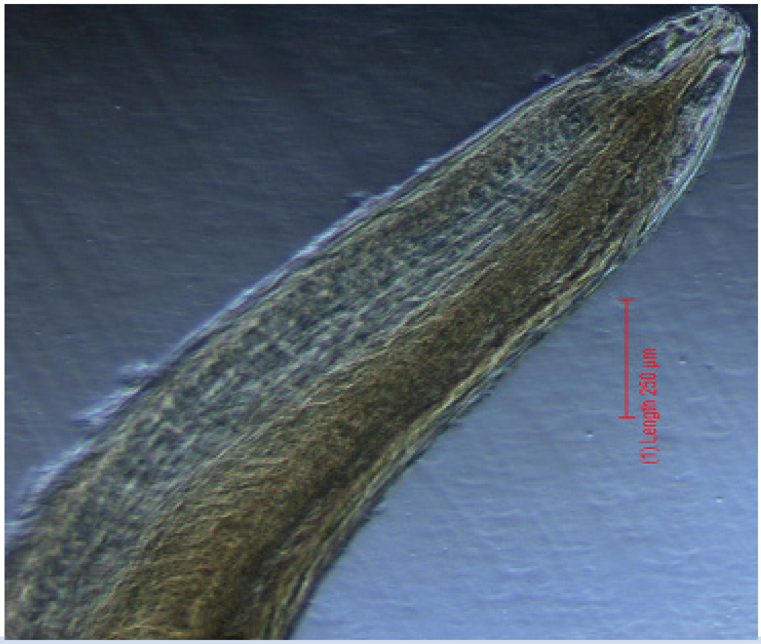

Figure 6. *Eustrongylides excicus* larvae anterior

Figure 7. *Eustrongylides excicus* larvae posterior

Figure 8. *Eustrongylides excicus* larvae in *Silurus glanis*

found to be infected with this nematode species and a total of 142 specimens were recorded in the host fish (Table 1). The second dominant parasite was *P. Homoion* (Figure 3). Eight of 13 fish were infected with this parasite (61.5 % prevalence). A total of 34 parasites were found in 8 host fish individuals (Table 1). A total of 11 specimens of *C. complanatum* metacercariae (Figure 4) were infected 7 of 13 fish examined with prevalence, mean intensity, and mean abundance of infection of 53.8 %, 1.5, and 0.8, respectively (Table 1). The Simav loach, *Oxynoemacheilus simavicus*, averaged 1.5g (± 0.3g, range 1.2 -2.3g) in weight and averaged 6.3cm (± 0.3cm, range 5.5 -6.7 cm) in total length. According to Table 1, a total of 6 parasitic individuals belonging to a nematode species, *Contracaecum rudolphii* B (Figure 1-2) were obtained from the host fish. We described this nematode species based on morphology and molecular analysis. These parasites were found to be localized in the abdominal cavity of the fish, with prevalence, mean intensity and mean abundance of 33.3%, 1 parasite/fish, and 0.3, respectively (Table 1).

The Angora loach, *Oxynoemacheilus angorae*, averaged 1.8g (± 0.7g, range 0.8 -3.1g) in weight and averaged 5.7cm (± 0.8cm, range 4.2-6.7 cm) in total length. As shown in Table 1, of the 12 fishes examined, only one species of nematodes were found in the abdominal cavity of 5 individuals of the host fish. Our detailed studies on morphological identification of the species identified as *Contracaecum rudolphii* B (Figure 1- 2) and we also confirmed with molecular analysis, as in the previous nematode species (in *O. simavicus*). Five fish individuals were infected with 8 parasite specimens. İnfection variables were recorded as follows: prevalence 41.6%, mean intensity 1.6 parasite/fish, and mean abundance 0.6 (Table 1).

The wels catfish, *Silurus glanis*, averaged 140.3g (± 100.1g, range 51 -320g) in weight, and averaged 25.5cm (± 7.5cm, range 16- 39.5 cm) in total length. A total of 5680 parasite individuals belonging to 2 helminth parasite species were recovered from the wels catfish.

Of these, *A. vistulensis* (Figure 5a,b,c) was found to be the most abundant compared to the remaining parasite species. A total of 5634 specimens of *A. vistulensis* were found in all 16 individuals of *S. glanis* examined (Table 1). The other parasite species in this fish is *E. excisus* larvae (Figure 6,7,8). A total of 46 specimens of this nematode species were found in the abdominal cavity of the host fish. The prevalence level of *E. excicus* larvae (Figure 6,7,8) was 56.2% and the mean intensity value was 5.1 parasite/fish (Table 1).

DISCUSSION

In this study, helminth parasites in three endemic fish and one native species from Susurluk basin, Turkey were investigated. Five helminth species were identified from these fishes; *Paradiplozoon homoion* (in *C. angorense*) and *Ancylodiscoides vistulensis* (in *S. glanis*) in the gills; *Clinostomum complanatum* metacercariae (in *C. angorense*) in the gills and gill cavities; *Contracaecum rudolphii* B (in *C.angorense*, *O. simavicus,* and *O. angorae*) and *Eustrongylides excicus* larvae (in *S. glanis*) in the abdominal cavity of the host fishes.

In the present study, two parasites, *P.homoion* and C. *rudolphii* B were identified using morphological and anatomic assessment, which confirmed molecular characterization. This study contributes to increasing the number of ichthyohelminthological studies on endemic fish and expanding our understanding of helminth diversity in *S.glanis* collecting data from a previously untouched locality in a different geographic region of Turkey. In addition to these findings, three endemic fish species are new hosts for helminth parasites recorded in them. At the same time, this study is the first ichthyohelmintological data for *S. glanis* living in Susurluk stream, Susurluk basin, Turkey. Therefore, the present study adds new data to the host range and the geographical distribution of parasite species recorded in this study.

The results show that *C. angorense* was found infecting with the highest number of helminth parasites (5). Moreover *S. glanis*, was found with a moderate number of parasites (2). *O. simavicus* and *O. Angorae* were found with the smallest number of parasite species.

Monogenean parasites *P. homoion* and digenean parasites *C. complanatum* metacercariae were only found in *C. angorense.* A total of 34 specimens of *P. homoion* were recovered from 8 of 34 *C. angorense* examined with prevalence and mean intensity of 61.53% and 4.2 respectively. It is also the most recorded species of *Paradiplozoon* in Turkey. *P. homoion* was previously reported five times from various freshwater fish species in the Marmara region conducted in our current study (Öztürk, 2005; Aydogdu, 2009; Aydogdu et al. 2020 a, b) found that prevalence and mean intensity values were 6;9.57%, 3.5, respectively in *Alburnoides manyasensis*. This prevalence and mean intensity values were nearly similar as compared to the finding of our current study.

As for *C. complanatum* metacercariae, a total of 11 specimens of this parasite were recorded from 7 of 13 individuals of *C. angorense*. This species has been previously reported from different fish species in a wide geographical area of Turkey: namely *Rhodeus 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* with prevalence values varying from 7.7 % to 53.8 % (Burgu et al.1988; Öge and Sarimehmetoglu 1996; Dávidová,, 2011;Çolak,2013;Soylu 2013;Şimşek 2018;Aydogdu,2020a). From these authors, Soylu [33] reported a 53.8% prevalence of *C. complanatum* metacercariae infecting *P. fluviatilis* from Lake Gala, the same as the value of our study.

In this study, the infection prevalence value of *C. rudolphiii*-B varied according to the host fish species. The highest prevalence value of this species was recorded in *C. angorense.* (100%) (Table 1), As for the infection value among *Oxynoemacheilus* spp, *O. angorae* is more infected by this species (41.6%) than *O. simavicus* (33.3%)(Table 1). According to the available literature, nematode species of the genus *Contracaecum* have been reported from various freshwater fish species living in different habitats in Turkey (Aydogdu,2002; Aydogdu,2008; Aydogdu,2011; Tekın-Özan,2005; Koyun and Altunel, 2007; Selver et al., 2009), nonetheless, neither of the parasite species was identified at a species level. To date, only one of the parasite species of this genus recorded in Turkey has been identified at the species level. It was determined as *C. rudolphi* by Innal et al. (2020) with morphological and anatomical features approaches. They reported this parasite species in *Carassius gibelio* from Karataş Lake, Burdur, Turkey. They determined a prevalence of 2.6% and a mean intensity of 27 parasites per fish in their study.

In the present study, the prevalence of *C. rudolphiii*-B varied from 100% in *C. angorense* from Susurluk stream, 41.6 % in *O. angorae,* and (33.3% in *O. simavicus* from Nilüfer stream. Surprisingly, contrary to their findings, we found that *C. rudolphii B* was heavily infected in individuals of three endemic fish. In addition to these findings, this study presents the first report on the molecular characterization of *C. rudolphiii* B from Turkey. Moreover, this study reports new host records and new localities were added for the distribution of this parasite species.

A. vistulensis was found to be the most prevalent and the most abundant compared to the remaining parasite in the understudy. *A. vistulensis* is a specific and very common parasite in European catfish, *S. glanis* living in different habitats in Europe and Asia. This parasite species has been previously reported from *Silurus glanis* distributed in different habitats across Turkey. namely from Serban Dam Lake, Sapanca Lake (Soylu, 1991; Soylu,2009); Terkos Lake (Soylu,2005);Almus Dam Lake (Turgut 2005);Durusu Lake (Soylu,2009), Siğirci Lake (Çolak, 2013) ;Sakarya River (Akmirza and Yardimci ,2014) and Büyükçekmece Dam Lake (Yardımcı et al.2018). *A. vistulensis* was recorded from *S. glanis* spreading in the wetlands of the same region where our current study was conducted in some of the above-mentioned studies. As to prevalence values of the infection of this species in *S. glanis* in Marmara Region: For example, this value was recorded at 42.6%, 25 % from Durusu Lake, Büyükçekmece Dam Lake, respectively. But we recorded prevalence values in Susurluk stream much higher (100%) than Durusu Lake and Büyükçekmece Dam Lake.

In the present study, *E. excisus* larvae were found in the abdominal cavity of *S. glanis* (Figure -1). This nematode species is a common parasite for several species of freshwater fishes distributed in different habitats across Turkey. This parasite has been previously reported in various fish species living in different habitats in Marmara Region and other regions from Turkey (Öztürk,2001; Öztürk,2002; Soylu 2005; Karatoy and Soylu, 2006; Aydogdu 2011; Çolak, 2013; Soylu, 2013; Akcimen, 2014;, Metin, 2014; Demir and Karakişi, 2016; Yardımcı et al., 2018; Innal et al., 2019). Among the above-mentioned authors, only two, Soylu (2005) and Yardimci et al. (2018) reported this parasite species in *S. galnis*. Soylu (2005) recorded this parasite species (10 samples) in *S. galnis* in Lake Durusu, but did not report any value for the prevalence of infection, while Yardimci et al. (2018) recorded 100 % the prevalence value in *S. glanis* from Büyükçekmece Dam Lake. Surprisingly, in our study, the prevalence of infection for this species was not close to or similar to the infection rates recorded in different fish species in most of the studies listed above while it was close to the prevalence value (46.2%) of infection of this species in Perca fluviatilis in only one study listed above (Çolak, 2013).

CONCLUSIONS

In this study, a total of 50 individuals of three endemic fish species and one native fish species were examined for helminth parasites between February 2021 and December -2021 from Nilüfer stream and Simav stream, in the northwest Anatolian region of Turkey. 5 parasite species were identified: the monogeneans *Ancylodiscoides vistulensis* and *Paradiplozoon homoion*, a digenean *Clinostomum complanatum* metacercariae and two nematodes, *Contracaecum rudolphiii*-B and *Eustrongylides excicus* larvae. The highest parasite community was recorded with monogenean parasite species.*A. vistulensis* was found to be the most prevalent parasite species. This study contributes to increasing the number of ichthyohelminthological studies on endemic fish species and to describe helminth species of *S. glanis* living in different geographic regions off Turkey. *Paradiplozoon homoion*, *Clinostomum complanatum* metacercariae, and *Contracaecum rudolphiii* B are reported for the first time parasitising these three endemic species. fish species (*Chondrostoma angorense*, *Oxynoemacheilus simavicus* and *Oxynoemacheilus angorae*) represent new host record for three helminth species adding new knowledge to its geographical distribution and host range. Finally, the present work is the first ichthyohelmintological study for *S. glanis* living in Susurluk stream, Susurluk basin, Turkey.

Declarations

Author' contributions

Nurten Aydogdu, Nesrin Emre and Ali Aydogdu contributed to the conception, coordination and design of the study. Nurten Aydogdu and Ali Aydogdu collected the samples. Nurten Aydogdu, Nesrin Emre and Ali Aydogdu performed the laboratory activities and organized the database. Nurten Aydoğdu, Nesrin Emre and Ali Aydoğdu conducted on morphological analysis studies for the identification of helminth parasite species. Nurten Aydogdu and Ali Aydogdu conceived of the study of diplozoid species by molecular analysis, and participated in its design and coordination and helped to confirm the morphological identity of diplozoid species by molecular analysis. Renato Aco-Alburqueque, Simonetta Mattiucci described nematode species, *Contracaecum rudolphiii* B based on morphology and molecular analysis. Nurten Aydogdu, Nesrin Emre and Ali Aydogdu conceived of the study, and participated in its design and coordination and helped to draft the manuscript. Renato Aco-Alburqueque and Simonetta Mattiucci critically oversaw the substantial revisions of the manuscript.

Conflicts of interest

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

Compliance with ethical standards

No ethical approval was required, as this study did not involve clinical trials or experimental procedure. During the study, no treatment/experiment was implemented on the live animal. All sampling and laboratorywork on fish have complied with the Republic of TurkeyMinistry of Agriculture and Forestry animal welfarelaws

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