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New host and distribution records for three species of *Dactylogyrus* (Dactylogyridae: Monogenea) from the endemic Anatolian fish species, *Alburnus escherichii* Steindachner, 1897 (Teleostei, Leuciscidae) in Turkey: Occurrence in relation to seasons and host factors

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Abstract

Background: This is the first record of dactylogyrid parasites from the endemic fish species, *Alburnus escherichii* Steindachner, 1897 in Turkey. **Aims:** The purpose of this study was to define the dactylogyrid specimens of *A. escherichii* from Sarısu stream. **Methods:** A total of 216 individuals of *A. escherichii* were collected from Sarısu stream between winter (February) and autumn (September) 2023 with seasonal intervals (one sample per season) and screened for the presence of dactylogyrid parasites infections. **Results:** It was determined that 57 *A. escherichii* were infected by one or more dactylogyrid parasite specimens (overall prevalence 26.3%). A total of 3 species of dactylogyrid parasites were identified on the gills: *Dactylogyrus fraterus* Wagener, 1909, *Dactylogyrus alatus* Linstow, 1878 and *Dactylogyrus minor* Wagener, 1857. *D. fraterus* was found to be the dominant parasite species in host fish species. **Conclusion:** To the best of our knowledge, this is the first study in the world on dactylogyrid parasites of *A. escherichii*. The host fish represents a new host record for each of the collected dactylogyrid parasite specimens. Furthermore, the present study provides the first record of *D. minor* in Turkish freshwater fish.

Key words: *Alburnus escherichii*, Dactylogyrid communities, Endemic fish, Geographical records, New host

Introduction

The freshwater ichthyofauna of Turkey consists of 427 species belonging to 20 orders, 37 families, and 97 genera. Among these, 21 species (4.9%) are alien and 215 species (50.4%) are considered endemic to Turkey (Çiçek *et al.*, 2023). One of these endemic species is *Alburnus escherichii* (Teleostei: Leuciscidae). Naturally, it is distributed in Akarçay, Sakarya, and Batı Karadeniz basins (Çiçek *et al.*, 2018, 2023) and was translocated to Konya Endorheic basin. Recently, it has been claimed to be also distributed in the Antalya basin (Küçük *et al.*, 2020). Only 106 of the 427 fish species distributed in Turkey's inland waters have been investigated, in which, a total of 183 species of helminth parasites were reported (Özer, 2022). Among the 183 helminth parasite species, 85 different monogenean parasite species were recorded, of which 47 species belong to the genus *Dactylogyrus* Diesing, 1850. In this context, regarding the host fish, to date, nine studies have been conducted on its ichthyoparasite fauna, yet none of the previous studies recorded dactylogyrid infection of the host fish (Özbek & Öztürk, 2010; Öztürk, 2011; Innal *et al.*, 2020; Öztürk,

2022; Nejat *et al.*, 2023). From these studies, more work is required for monitoring the dactylogyrids species of the host fish as very few studies have been carried out so far. The important monogenean parasites of fishes in Turkey are poorly known and require further studies to fill the gap. Therefore, the present study aimed to provide the first data on the dactylogyrid monogeneans fauna of this host fish in the inland waters of Turkey to determine how the prevalence, abundance and intensity of clinostomid infection varies according to seasons, as well as with the size and sex of the host fish.

Materials and Methods

Ethical standard

No ethical approval was required, as this study did not involve clinical trials or experimental procedures. During the study, no treatment/experiment was implemented on live animals. All sampling and laboratory work on fish have complied with the laws of Ministry of Agriculture and Forestry Animal Welfare, Republic of Turkey.

The study area, Sarısu stream (Eskişehir), is an

important water source with warm water resources and feeds the Porsuk stream. The flow rate of the water is quite slow and stagnant (Aksu *et al.*, 2021). The sampling point is 1 km from the water source and the water surface is muddy (Emiroğlu *et al.*, 2016) (Fig. 1). A total of 216 individuals of *Alburnus escherichii* Steindachner, 1897 were collected from Sarisu stream (Eskişehir) (Fig. 1) between winter (February) and autumn (September) 2023, with seasonal intervals (one sample per season). The seasonal samples of fish specimens were collected using electrofishing (Samus 1000). A total of 41 to 70 specimens of *A. escherichii* were collected during each sampling campaign. The fish were placed in plastic tanks aerated with oxygen and immediately submitted alive for parasitological investigation at the research laboratory, Faculty of Veterinary Medicine, Bursa Uludag University. They were kept in aerated 20-litre aquaria and examined several days later. Fish specimens were killed by severing the spinal cord posterior to the cranium. At necropsy, individual fish were measured and divided into two groups based on their length, the first group ranging from 6.00 to 9.99 cm and the second from 10.00 to 14.00 cm. The sex of each fish was determined upon dissection; 116 were females and 100 were males (Table 1). During dissection, the gills of each individual were separated, kept moist in petri dishes containing physiological water and examined under stereo-microscopes (SMZ 745 Nikon and SZ61 Olympus) for the detection of monogenean parasites. Of the found parasites, three specimens of monogenea were collected and preserved in 70% ethanol for use in future studies if needed; permanent preparations were made using glycerine ammonium picrate (Malmberg, 1957). During the study period, the collection date of each monogenea species was classified according to the sampling season

and the sex of the host. Similarly, data were divided into two groups based on the length of each host fish (Table 1).

All dactylogyrids specimens were identified using standard taxonomic criteria related to the hard parts of their haptor and reproductive organs, as described by Markevic (1951), and Gussev (1985). Parasitological terms (prevalence, mean intensity and abundance) were calculated according to Bush *et al.* (1997). The prevalence rate was obtained using the formula:

Prevalence% = (Number of hosts infected/Total number of hosts examined)*100

Mean intensity = Total number of parasite of a particular species found in samples/Number of hosts infected with that parasite

Mean abundance = Total number of individual parasites of a particular species found/Total number of hosts of that species examined (including both infected and uninfected hosts)

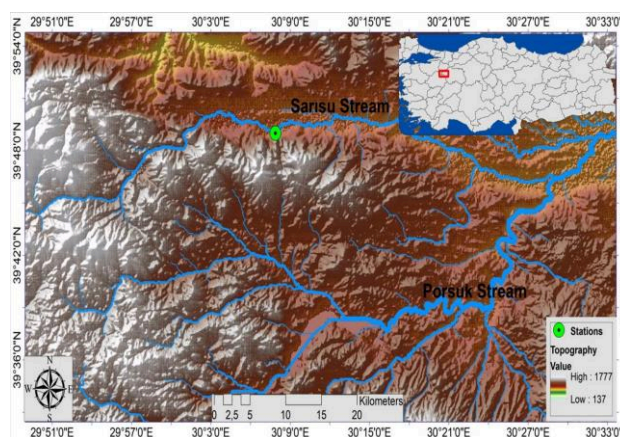


Fig. 1: Sampling locality of *Alburnus escherichii* in the Sarisu stream in Turkey

Table 1: Distribution of *Dactylogyrus* spp. infection values in *Alburnus escherichii* from Sarisu Stream, Eskişehir according to fish length and sex

		Examined fish number	Infection parameters				
			Infected fish number	Prevalence (%)	Mean intensity ±SD	Mean abundance	Intensity range (min-max)
<i>Dactylogyrus fraternus</i>	Fish sex groups						
	Female	116	14	12.1	2.71±4.23	0.33	1-17
	Male	100	33	33	2.67±2.16	0.88	1-9
	Fish length (cm)						
	6-9.90	127	29	22.8	2.93±3.28	0.67	1-17
	10-14	89	18	20.2	2.28±2.10	0.46	1-9
<i>Dactylogyrus minor</i>	Fish sex groups						
	Female	116	4	3.44	2.25±1.89	0.07	1-5
	Male	100	9	9	1.66±0.92	0.15	1-3
	Fish length (cm)						
	6-9.90	127	5	3.93	2±1.73	0.07	1-5
	10-14	89	8	8.98	1.75±0.96	0.15	1-3
<i>Dactylogyrus alatus</i>	Fish sex groups						
	Female	116	8	6.9	2.12±3.18	0.15	1-10
	Male	100	10	10	1.70±1.05	0.17	1-4
	Fish length (cm)						
	6-9.90	127	13	10.2	2.23±2.52	0.23	1-10
	10-14	89	5	5.6	1±0.00	0.06	

The standard statistical computation (standard deviation) was carried out using Microsoft Excel (Office 2000). Concordance of the continuous data to normal distribution was tested by Kolmogorov-Smirnov test. Kruskal-Wallis (more than two groups) test was applied to find significant differences in the average density of parasite species according to seasons the Mann-Whitney U test was used. The correlations among the continuous variables were determined by Spearman correlation test according to the distribution of the variable. All statistics analyses were performed using IBM SPSS Statistics v. 28 for Windows. The significance level of $\alpha \leq 0.05$ was used.

Results

In this study, dactylogyrid monogeneans of *Alburnus escherichii* (Teleostei: Leuciscidae) from Sarisu stream (Eskişehir) were investigated between winter 2023 and autumn 2023 with seasonal intervals. Among the 216 individual of *A. escherichii* examined, the gills of 47 host samples were infected with one or two dactylogyrid monogenean species. Overall 21.7% of the fish were infected. According to the hard parts of the haptor and the reproductive organs observed in the collected parasite samples, three monogenean species were identified, i.e. *Dactylogyrus fraternus* (Wagener, 1909) (Figs. 2a, b), *Dactylogyrus alatus* (Linstow, 1878) (Figs. 3a, b) and *Dactylogyrus minor* (Wagener, 1857) (Figs. 4a, b). As for the general infection values of dactylogyrid specimens detected, *D. fraternus* was found to be the dominant parasite species in the host fish. A total of 126 specimens of *D. fraternus* were observed in 47 out of the 216 fish examined. Its mean intensity of infection was found to be 2.68 ± 2.88 parasite/fish. *D. alatus* was identified as the second most dominant parasite, as it was found in 18 out of 216 examined hosts, with a prevalence and a mean intensity of infection of 8.3% and 1.88 ± 2.19 , respectively. A total of 34 parasites were found in 18 fish. Additionally, 24 specimens of *D. minor* were recovered from 13 *A. escherichii* (prevalence: 6.01% and mean intensity: 1.84 ± 1.24 parasite/fish).

Seasonal differences in infection parameters are shown in Table 2. During this study, two dactylogyrid parasite species were recorded in host fish in all sampling seasons, whereas *D. minor* was not detected in

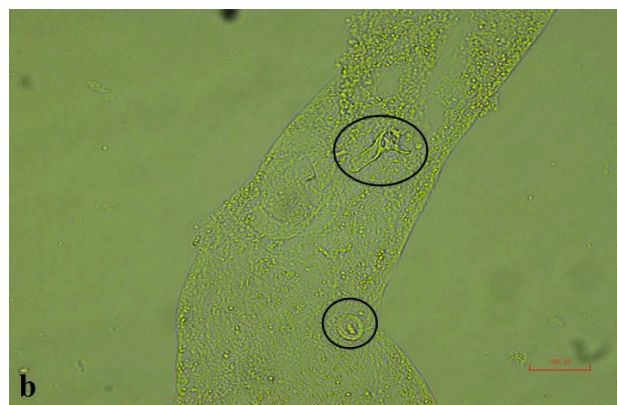


Fig. 2: *Dactylogyrus fraternus* (a) haptor $\times 40$ (scale bar, 40 μm), and (b) copulatory organ and vaginal tube $\times 100$ (scale bar, 100 μm)



Fig. 3: *Dactylogyrus alatus* (a) haptor $\times 100$, and (b) copulatory organ $\times 100$ (scale bar, 100 μm)

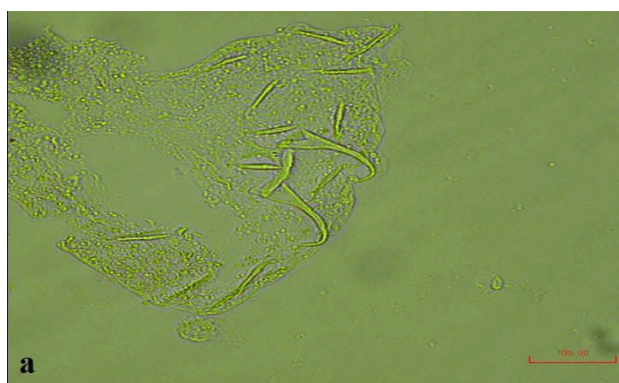




Fig. 4: *Dactylogyrus minor* (a) haptor $\times 40$ (scale bar, 40 μm), and (b) copulatory organ and vaginal tube $\times 100$ (scale bar, 100 μm)

the summer samples. Of all specimens, *D. fraternus* appeared in high numbers. Its seasonal prevalence varied from 5.4% to 35.7%, highest in spring and lowest in summer (Table 1). The abundance (ranged from 0.07 to 1.01) was also highest in spring. The mean intensity of this parasite ranged between 1.33 ± 0.57 and 3.33 ± 2.3 parasite/fish, highest in autumn samples (Table 1). The significance of the difference per season was calculated using the Mann Whitney U test which showed a significant difference between the infection in summer and winter ($P=0.003$, $U=1084$), spring and summer ($P=0.001$, $U=1349$) and when spring and autumn ($P=0.023$, $U=1142$).

In the case of *D. alatus*, the highest seasonal prevalence of infection was recorded in spring, while the highest abundance and mean intensity values were recorded in winter. Differences in the number of parasites detected in each season were calculated using

the Kruskal Wallis H test. However, there was no significant correlation between the seasons and the numbers of this parasite species ($P=0.061$).

A pattern similar to that of *D. alatus* in fluctuations of infection parameters was also noted with *D. minor* in the present study. The prevalence of infection was the highest in spring as indicated in Table 1, while the highest mean intensity was during winter. Accordingly, *D. minor* was noted the lowest number on the host fish's gills (Table 1). There was a significant difference between the infection in spring and summer ($P=0.003$, $U=1680$) and between spring and autumn ($P=0.041$, $U=1262$).

The prevalence, intensity and abundance levels of the three dactylogyrid specimens according to the length and sex of the host fish are presented in Table 2. As shown in Table 2, a higher prevalence was observed for *D. fraternus* (22.8%) in fish of 6-9.90 cm and for *D. alatus* (10.2%) and *D. minor* (8.7%) in fish of 10-14 cm long. Similarly, the mean intensity of *D. minor* and *D. alatus* reached its maximum level (2 ± 1.73 and 2.23 ± 2.52 , respectively) in fish of 6-9.90 cm long.

The prevalence and abundance of infection of the three dactylogyrid species were higher in males as compared with females, while the mean intensity of infection was higher in female fish (Table 2). Additionally, while there was a statistically significant difference in the number of *D. fraternus* in male host fish (Mann-Whitney U-test=4540.5, $P<0.001$), no significance was observed for *D. alatus* and *D. minor* ($P=0.268$ and 0.089, respectively).

According to Spearman test, a positive correlation was only observed between the length of *A. escherichii* and the number of *D. minor* ($P=0.009$, $r=0.150$) (Table 3).

Table 2: Distribution of *Dactylogyrus* spp. infection values in *Alburnus escherichii* from Sarısu Stream, Eskişehir according to seasons

<i>Dactylogyrus</i> species in <i>A. escherichii</i>	Seasons	Examined fish number	Infection parameters				
			Infected fish number	Prevalence (%)	Mean intensity $\pm$ SD	Mean abundance	Intensity range (min-max)
<i>Dactylogyrus fraternus</i>	Winter	49	13	26.5	2.38 ± 4.42	0.63	1-17
	Spring	70	45	35.7	2.84 ± 2.09	1.01	1-9
	Summer	56	3	5.4	1.33 ± 0.57	0.07	1-2
	Autumn	41	6	14.6	3.33 ± 2.30	0.49	1-8
<i>Dactylogyrus minor</i>	Winter	47	2	4.08	3 ± 4.29	0.04	1-5
	Spring	70	10	14.3	1.70 ± 0.67	0.24	1-3
	Summer	56	0	0	0	0	0
	Autumn	41	1	2.4	1	0.02	1
<i>Dactylogyrus alatus</i>	Winter	49	4	8.2	3.25 ± 4.47	0.27	1-10
	Spring	70	9	12.9	1.56 ± 1.01	0.20	1-4
	Summer	56	1	1.8	1	0.02	1
	Autumn	41	4	9.8	1.50 ± 1	0.15	1-3

Table 3: Correlation between the number of 3 different *Dactylogyrus* species found in *Alburnus escherichii* and fish length

<i>Dactylogyrus</i> species in <i>A. escherichii</i>	<i>Alburnus escherichii</i> of length	
	r	P-value
<i>D. fraternus</i>	108	0.113
<i>D. minor</i>	150	0.009
<i>D. alatus</i>	034	0.618

Discussion

No research has been conducted on the incidence of monogenean parasites in the endemic Anatolian fish species, *A. escherichii*. This study is the first parasitological investigation of this fish species in Turkey. Three monogenean species were identified, i.e., *Dactylogyrus fraternus* (Figs. 2a, b), *Dactylogyrus alatus* (Figs. 3a, b) and *Dactylogyrus minor* (Figs. 4a, b). The difference consists in the shape of the vaginal tube and the chitinoidelements of the adhesive disc. The examined fish species is a new host for all these dactylogyrid species. Therefore, the present study adds new data to the geographical distribution and the host range of these dactylogyrid specimens. Moreover, *D. minor* is reported for the first time in Turkey.

In this study, *D. fraternus* was the most frequent monogenean in the host examined (21.8% of all examined fish) and was also found to be the most numerous parasite species, with a total of 126 individuals recorded from 47 fish. This dactylogyrid species is very common in fish species distributed everywhere in the genus *Alburnus* (Gusseu, 1985; Šimková *et al.*, 2001; Stojanovski *et al.*, 2009; Suleymanova, 2023; Suleymanova and Ali, 2023). In accordance with this information, to our knowledge, *D. fraternus* has been previously reported in four fish species living in different habitats in Turkey (Aydoğdu and Selver, 2006; Koyun and Altunel, 2007; Koyun, 2011). According to the above data, different prevalence and mean intensity values have been reported. For example, Aydoğdu and Selver (2006) found the prevalence and mean intensity of 58.3% and 3.4, respectively. Koyun (2011) reported this parasite as a dominant species in *A. alburnus* with a prevalence of 49.6% and a mean intensity of 5.20 in Porsuk River. In this context, while the prevalence value of *D. fraternus* infection in this study is not compatible with the above studies, the mean intensity value of this species is similar to the findings of Aydoğdu and Selver (2006). The conclusion is that the similarity in parasite fauna and infection rates among host fish living in similar habitats can be explained by host-parasite specificity.

With regard to *D. minor*, there are no studies on the incidence of this species in fish in Turkey. Stojanovski *et al.* (2009) recorded this species from *A. alborella* in Lake Ohrid, Macedonia, with a prevalence of 31.53% and a mean intensity of 4.16. They also reported that *D. minor* infected *A. alburnus belvica* living in Lake Prespa, with a prevalence of 17.24% and a mean intensity of 5. Thus, it

can be seen that the prevalence and the mean intensity of infection with *Dactylogyrus* spp. differs according to the host species and locality. Variations in the prevalence and mean intensity of *Dactylogyrus* spp. infections in freshwater fishes might be influenced by various factors, such as the parasite and its life cycle, host specificity, preference, species, hormonal status, immunological response and migration (Hanzelova and Zitnan, 1985; Šimková *et al.*, 2005; Koyun *et al.*, 2015).

Seasonal variation of infection rates of dactylogyrid species in *Alburnus* spp. have been studied in Turkey. For example, Koyun (2011) showed that the prevalence of infection with *D. fraternus* and *D. alatus* in *A. alburnus* from Porsuk River was higher during summer (69.5%, 65% respectively). Additionally, in the same study, the author recorded the highest mean intensity level of *D. fraternus* in the summer sampling (5.10), while the highest level of mean intensity was recorded in spring sampling for *D. alatus* (3.50). Similarly, Koyun (2001) noticed that *A. alburnus* in Enne Dam Lake was highly infected with *D. fraternus* and *D. alatus* during summer (prevalence 100%, 76%, respectively). Contrary to these findings Tunç and Koyun (2018) recorded the highest infection prevalence value of *D. alatus* in the autumn and the winter seasons in the Mosul Bleak fish, *A. mossulensis*. Generally, it seems that the rate of parasitic species of this genus is high in spring and early summer. This was explained based on the fact that during the reproduction, the eggs are laid directly into the water, the oncomiracidium hatch out, settle on the host fishes and reach sexual maturity during these seasons when the water temperature was the highest. Temperature is generally considered one of the most important factors determining the seasonal occurrence and abundance of dactylogyrid parasites (Koskivaara *et al.*, 1991).

In this study, the prevalence of *D. fraternus* and *D. alatus* was highest in 6 to 9.90 cm fish. However, *D. minor* was more commonly identified in fish with lengths of 10 to 14 cm (Table 2). As for the mean intensity, its highest level for the three *Dactylogyrus* species was observed in fish from 6 to 9.90 cm long. There was no correlation between the length of *A. escherichii* and the number of the three *Dactylogyrus* (Table 3). Neary *et al.* (2012) found that *D. alatus* infection value was slightly higher in *A. orontis* on medium length groups than on the other length groups. Further, Koyun and Altunel (2007) have demonstrated that there is no relationship between the numbers of *D. fraternus* and *D. alatus* and the length of *A. alburnus*. In general, a positive relationship exists between the total number of dactylogyrid parasite specimens per host and the host length. This has been illustrated by several researches (Aydoğdu *et al.*, 2003; Özer and Öztürk, 2005; Šimková *et al.*, 2005; Öztürk and Altunel, 2006; Abdullah, 2007; Koyun and Altunel, 2007; Öztürk, 2014; Aydoğdu *et al.*, 2015; Abdullah and Nasraddin, 2020). They found that the infection of *Dactylogyrus* spp. increases with the length of host fishes. On the other hand, there are studies (Mhaisen and Abdullah, 2006;

Aydoğdu and Kubilay, 2017) that have found opposite results, detecting highest infection prevalence and mean intensity values of *Dactylogyrus* spp. in young fish. Further, Selver *et al.* (2009) and Neary *et al.* (2012) have demonstrated no relationship between the length of the host fish and the infection values of *Dactylogyrus*. The above authors who found a positive correlation between increased fish size and Dactylogyrids infestation attribute this to the fact that the larger-sized host likely had a larger gill surface for infection that the volumetric water passing through their gills carried more oncomiracidium, the larger fish had more space for parasite attachment along with more time to accumulate the parasite, and an increase in gill surface is associated with increased length. On the other hand, some of the authors claim that small fish have no permanent immunity against these parasites, and their antibody response is slower compared with larger fish, leading to higher accumulation of parasites on their surfaces.

In our study, the prevalence and abundance levels of infection of the three dactylogyrid species were higher in males compared with females (Table 2). In Turkey, Öztürk (2014) found higher prevalence of *D. vistulae* in male individuals of the chub, *Squalius cephalus* (L. 1758). Similarly, Elbay and Öztürk (2021) found that *D. vistulae* heavily infected male individuals of *Squalius recurvirostris*. In the opposite of these findings, Tunç and Koyun (2018) have found higher *D. alatus* infection in female individuals of *A. mossulensis*. In contrast, Mhaisen and Abdullah (2006), Abdullah (2007), and Abdullah and Nasraddin (2020) found no host sex related effects on the infection of dactylogyrid in different fish specimens. The authors above concluded that this was due to mucus, colour, hormonal status, physiological state of fishes, differences in food behaviour and morphological differences between both sexes that could lead to one sex being more parasitized with dactylogyrid than the other.

To the best of our knowledge, the present study is the first report on dactylogyrid specimens from the endemic Anatolian fish species, Caucasian bleak *A. escherichii* in Turkey. Additionally, *D. minor* was newly identified, raising the number of dactylogyrid species recorded in Turkey to 48. This study adds a new locality for the distribution of three dactylogyrid specimens. It also provides further insight into how infection parameters of these species vary according to seasons, host fish length and sex.

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Conflict of interest

The author declares no competing interests.

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