

Some Parasitological Features of *Paradiplozoon bliccae* (Monogenea: Diplozoidae) Infestation in *Pseudophoxinus burduricus* - An Endemic Fish Species from Doğanbaba Creek (Lake Salda, Turkey)

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Research Article

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Abstract

This study was aimed to identify *Paradiplozoon bliccae* (Monogenea: Diplozoidae) in an endemic fish species *Pseudophoxinus burduricus* (Teleostei: Cyprinidae) and to describe parasite's prevalence and intensity of infection. *P. burduricus* specimens were collected between January 2014 and February 2015 in Doğanbaba Creek (Yeşilova-Burdur). A total of 60 *P. burduricus* specimens were examined for monogenean parasites. During the course of surveys, diplozoid specimens were collected from the gills of these fish and examined microscopically for identification. *Paradiplozoon bliccae* specimens were isolated from the examined fish. Prevalence, intensity, and seasonality of infection, age classes, and sex compositions of the infected population have been calculated. It has been recorded that the highest point of prevalence being in summer and the highest invasion value in the two-age class of fish. Hyperaemia, haemorrhage, and atrophy of the gills were observed during pathological examination of the parasite infested fish individuals.

Keywords: Monogenea, Diplozoidae, Burdur, endemic

Doğanbaba Çayı (Salda Gölü, Türkiye)'nın Endemik balık türü *Pseudophoxinus burduricus*'ta *Paradiplozoon bliccae* (Monogenea: Diplozoidae) Enfeksiyonunun Bazı Parazitolojik Özellikleri

Özet

Bu çalışmada endemik bir balık türü olan *Pseudophoxinus burduricus* (Teleostei: Cyprinidae) 'da bulunan *Paradiplozoon bliccae* (Monogenea: Diplozoidae) bireylerinin tanımlanması ve parazitin enfeksiyon prevalansının ve yoğunluğunun belirlenmesi amaçlanmıştır. *P. burduricus* örnekleri Ocak 2014 ile Şubat 2015 arasında Doğanbaba Deresi'nden (Yeşilova-Burdur) toplanmıştır. Monogenean parazitleri açısından toplam 60 *P. burduricus* örneği incelenmiştir. Balık solungaçlarından diplozoid örnekleri toplanmış ve tespit için mikroskopik olarak incelenmiştir. *Paradiplozoon bliccae* örnekleri balıklardan izole edilerek enfeksiyon prevalansı, şiddeti ve mevsimselliği, yaş sınıfları ve enfekte olmuş popülasyonun cinsiyet kompozisyonları hesaplanmıştır. En yüksek prevalans değeri yaz mevsiminde ve en yüksek istila değeri iki yaş balık sınıfında kaydedilmiştir. Parazit bulaşmış balık bireylerinin patolojik olarak incelenmesi ile solungaçlarda hemoraji, solungaç atrofisi gözlenmiştir.

Anahtar Kelimeler: Monogenea, Diplozoidae, Burdur, endemik

INTRODUCTION

Monogenea is a diverse group of ectoparasites most of which predominantly live on the gills, skin arches, and fins of a fish. A few may invade the rectal cavity, ureter, body cavity, and even the blood vascular system. World widely about 4,000 to 5,000 species of monogeneans have been described on fishes from freshwater and marine systems also at different temperature ranges (Reed et al., 2009a; Reed et al., 2009b).

Monogenean parasites transmit to another host via contagion and many of them are host specific. They have no intermediate hosts; new hosts are infected by tiny free-swimming ciliated larvae or oncomiracidia (Reed et al., 2009a; Kearns, 2011; Kearns, 2014; Bommakanti, 2016).

Diplozooids (subfamily Diplozoinae) as blood-feeding ectoparasites have a unique position in monogeneans with having unusual body morphology and a direct life cycle. They invade mainly the gills of cyprinid fishes. The life cycle includes permanent fusion of two larval forms that later turn into a single worm. Adults have the specific hook-like structure, use two haptors with four pairs of clamps to attach to fish gills, and create a X-shaped body (Pečínková et al., 2007; Hodová et al., 2018). Infected gills are pale, bloody, damaged and with excessive mucous secretion resulting in decreased gill respiratory function and mortality (Reed et al., 2009b; Al-Nasiri, 2010).

It is known that diplozoons infect a large number of fish species in Europe (Matějusová et al., 2001; Matějusová et al., 2004; Stavrescu-Bedivan and Aioanei, 2008; Stojanovski et al., 2010; Konstanžová et al., 2017) in Asia (Soylu, 2009; Ahmad et al., 2015; Al-Nasiri and Balbuena, 2016; Jirsová et al., 2018) and Africa (Dos Santos et al., 2015; Gilbert and Avenant-Oldewage, 2016a-b).

P. bliccae belongs to the *Paridiplozoon* genus from the Diplozoidea family. Several parasitological studies have been conducted on monogenean parasites in Turkey (Özer et al., 2004; Öztürk and Altunel, 2006; Soylu, 2009; Koyun, 2011; Soylu, 2012) and only a few *Paradiplozoon* species were reported.

Paradiplozoon homonion had been previously reported by Soylu and Emre (2007) from *Pseudophoxinus antalyae* and *Cyprinus carpio* in Kepez Pond; by Koyun and Altunel (2007) from *Alburnus alburnus* in Enne Dam Lake; by Öztürk (2011) from *Rutilus rutilus* in Manyas Lake; by Akmirza and Yardimci (2014) from *Abramis brama* and *Blicca bjoerkna* in Sakarya river; by Öztürk and Özer (2014) from *Scardinius erythrophthalmus* in Lower Kizilirmak and by Altan and Soylu (2018) in *Blicca bjoerkna* from Akgöl Lake.

Aydoğdu et al. (2001) found *P. megan* from *Squalius cephalus* in Doğançı Dam lake. *P. megan* was also reported in Susurluk Stream (Gürkan and Tekin-Ozan, 2012). *P. bingolensis* was described as a new species of genus in *Garra rufa* from Murat River (Civáňová et al., 2013). *P. bliccae* has been firstly reported from *Squalius fellowesii* in Doğanbaba creek (Ünal et al., 2017).

Pseudophoxinus is represented by 18 species in Anatolia, where an important diversification center for this genus is. Except for two of this species, all of them are endemic to Anatolia (Küçük et al., 2012; Küçük et al. 2013). Burdur spring minnow *P. burduricus* (Teleostei, Cyprinidae) is an endemic fish species of Turkey. It is known from several freshwater systems in Central Anatolia and in the surroundings of Lake Salda and Lake Burdur basins; Karamanlı stream, Düğer spring, Değirmendere stream, Sazak spring near Lake Yarışlı. The conservation status of the fish has been referred to category E - endangered fish (Freyhof, 2014).

There is a limited number of studies on the biological features, distribution (Küçük et al., 2012; Gülle et al., 2016), identification (Küçük et al., 2013), and length-weight relationship (Yoğurtçuoğlu et al., 2016, İlhan and Gücer, 2018) of *P. burduricus*. And except the Innal et al. (2017), there are no data related to the parasites on *P. burduricus*.

The parasites in threatened fish species need to be investigated for the management and conservation of fish populations in natural waters (Aydoğdu et al., 2015). As mentioned above, Innal et al. (2017) recorded *Lerneae cyprinacea* with the highest prevalence of 28.3 % on *P. burduricus* from Düğer Spring Creek (Burdur-Turkey). The objective of this work was to investigate parasite infection of gill from *P. burduricus* in Doğanbaba Creek (Yeşilova-Burdur).

MATERIALS and METHODS

Fish sampling and dissection

Pseudophoxinus burduricus specimens were collected by electrofishing method in Doğanbaba Creek (37°35'48.35"N, 29°38'24.89" E) from January 2014 to February 2015. A total of 60 *P. burduricus* were investigated for the presence of parasite specimens. Fish specimens were transported in an oxygenated 20-litre aquarium filled with stream water. They were maintained in the aquarium for 2-3 hours and subsequently anaesthetized by MS-222. Following a standard parasitological method for the detection of diplozoid species, all *P. bliccae* specimens were removed from the gills. Monogeneans were isolated in the laboratory using a dissecting needle and stereomicroscope. To evaluate pathology, gills samples infected with the parasites were fixed in 10% neutral formalin and routinely processed.

Five micron sectioned and stained with haematoxylin eosin. The gills were examined before and after the removal of the parasites. Infected gills of fishes were examined under microscope. Fish age and sex were noted. Fish age was determined by using method of Lagler (1956). Fish were dissected and classified according to sexual states (immature, mature, female, and male). Prevalence, intensity, and seasonality of infection, age classes, and sex compositions of the infected population have been investigated. Prevalence (Pr %), as the percentage of hosts infected with a particular parasite species or taxonomic group and intensity (Int), as the number of individuals of a parasite species in/ on a single infected host was calculated following Bush et al. (1997). A Kruskal–Wallis test was applied to find significant differences in the prevalence of the parasite species for host fish size, ages, and seasons. The differences in parameters were considered significant at $P < 0.05$.

RESULTS

This study presents the first investigation of the monogenean fauna occurring on the gills of endemic *Pseudophoxinus burduricus* species inhabiting Doğanbaba Creek. The monogenean parasite *Paradiplozoon bliccae* was found on 14 of 60 *P. burduricus* (23.33 %). A total of 86 specimens were recovered from gills of 14 fish. Prevalence and intensity of infection in different age groups and sex classes of fish species are shown in Table 1 and Table 2, respectively. Seasonal variation of infection and mean intensity are shown in Table 3.

Four age classes were determined in captured specimens of *P. burduricus*. The age group I was dominant in the population with 35 individuals and 5 of them were infected. The prevalence of infection and infection intensity was higher among age group II. There were significant differences ($p < 0.05$) in the infection rates of *P. bliccae* between ages. No diplozoon was found in juvenile individuals and the group of age III.

Table 1. Prevalence and intensity of infection among different age groups of *P. burduricus*

Ages	N	N'	P	Total	Int.
0	6	0	0	0	0
I	35	5	14.29	24	4.8
II	17	9	52.94	62	6.89
III	2	0	0	0	0
Total	60	14	23.33	86	6.14

0 – I – II – III – age categories, N – total number of fish in the age category, N' – Number of infected fish, P Prevalence (%), Total – number of recovered parasites, Int. – intensity of infection

Among examined population of *P. burduricus*, male individuals had superiority in numbers (35 from 60). But female hosts had higher levels of infection. Among all infected fishes, 8 were female, and 6 were male. The prevalence rates of female and male individuals are 42.11 % and 17.14 %, respectively. The differences in *P. bliccae* parasitic infections and the sex of the fish hosts is statistically significant ($p < 0.05$). Results are presented in Table 2.

Table 2. Prevalence of infection in sex groups of *P. burduricus*

Sex	N	N'	P
Immature	6	0	0
Female	19	8	42.11
Male	35	6	17.14
Total	60	14	23.33

N – total number of fish, N' – Number of infected fish, P – Prevalence (%)

The seasonal prevalence and intensity related to *P. bliccae* infection are shown in Table 3. Prevalence value was highest (45.45 %) in the summer and lowest (0 %) in autumn and it increased in winter (25.93 %). There were significant differences ($p < 0.05$) in the infection rates of *P. bliccae* between seasons.

Table 3. Seasonality of infection and mean infection intensity for *P. bliccae* in *P. burduricus*

Season	N	N ⁱ	P	Total	Int.
Spring	27	7	25.93	36	5.14
Summer	11	5	45.45	27	5.4
Autumn	4	0	0	0	0
Winter	18	2	11.11	23	11.5
Total	60	14	23.33	86	6.14

N – total number of fish, Nⁱ – Number of infected fish, P – Prevalence (%), Total – number of recovered parasites, Int. – intensity of infection

Mean intensity reached a peak in winter (11.50 %), but then decreased in spring and it fell to the lowest level in autumn (0 %). The total number of *P. bliccae* was 86 whereas the maximum abundance was recorded during spring (36) and the minimum values were obtained during autumn (0); in winter, there were found 23 individual on only two host specimens.

Gross examination of the gills of *P. burduricus* revealed *P. bliccae* attached to the gills. Parasites were generally localised at filament extremities and caused irregular appearance at the gills. More than one parasite was observed on the same fish. Marked swelling, hyperaemia, and in some case haemorrhages were observed at the gills that parasites attached area. Besides, atrophy was noticed the filaments after removal of the parasites (Figure 1). Microscopical examination revealed desquamation at the epithelial layer of the gills. Hyperaemia, haemorrhage and oedema were common histopathological findings at the parasite attachment site. In some cases slight to moderate leukocyte inflammation at these areas.



Figure 1. (A) Slight hyperaemia, haemorrhage (white arrow), and irregular appearance of the gills after removing the parasites (black arrow), (B) macro appearance of the parasites that collected from the gills, (C) attached parasite (arrow) on the gill

DISCUSSION

Monogenea is a group of species-rich parasitic flatworms consisting of many described species that are commonly found on fishes and lower aquatic invertebrates. Due to having their high-degree host specificity, parasitic monogeneans are used as excellent biological indicators to unveil host-species relationships (Kmentová et al., 2016). The presence of monogeneans is regarded as a sensitive biological indicator for the health situation of their habitat (Stojanovski et al., 2009).

In this study, monegean parasite *P. bliccae* was found in *P. burduricus*. This is a typical blood-feeding ectoparasite from Diplozoidae family (Polyopisthocotylea, Monogenea) which have an obvious specificity for host species. *P. bliccae* specimens were reported from some different fish species in Europe and Asia (Matějusková et al., 2001; Al-Nasiri, 2009; Sobecka et al., 2014). Ünal et al. (2017) recorded *Paradiplozoon bliccae* for Turkey parasite fauna firstly from the gills of *Squalius fellowesii* (Cyprinidae) in Doğanbaba Creek (Yeşilova-Burdur).

Herein, a total of 86 parasites attached to the gill of their hosts were obtained from 14 infected fish specimens with a prevalence 23.3 % and quite high mean intensity of infection 6.14. According to the data collected so far, different infectious densities were detected in different fish species. Ünal et al. (2017) found that prevalence and mean intensity were 28.3 % and 3, respectively in *Squalius fellowesii*. Al-Nasiri (2009) recorded *P. bliccae* with their percentage incidence of infection: from *Cyprinion macrostomum* (13.7 %) and *Cyprinus carpio* (18.2 %) in Tigris river passing through Tikreet city,

Salah Al-Deen province. Sobecka et al. (2014) reported *P. bliccae* from *Leuciscus idus* in Lake Dabie, Poland with prevalence of 1.81 % and mean intensity of 1.50.

Many factors affect the prevalence of paradiplozoon infection in freshwater fishes. There are several studies that reveal these relations (Koyun and Altunel, 2007; Aydoğdu et al., 2008; Tombi et al., 2014; Gilbert and Avenant-Oldewage, 2016b).

Of 60 *P. burduricus* classified according sex combination, 35 male and 19 female fish specimens, carried 6 and 8 *P. bliccae*, respectively. There was no infection in any of 6 immature individuals. Prevalence was found to be higher in females (42.11 %) than in the males (17.14 %). Contrary of these results, male dominance in infection was reported in studies of Tunç and Koyun (2018) about *P. homoion* in *Alburnus mossulensis* (47.61 %) and Ünal et al. (2017) concerning *P. bliccae* (52.9 %) in *S. fellowesii*.

The ages of examined specimens ranged from 0 to III and total prevalence was 6.14 %. The one age class showed dominance in the population (35 specimens). In our study highest prevalence is reported to be 52.94 % in the two age class while in Ünal et al. (2017) maximum prevalence were reported in the four-age group of *Squalius fellowesii* (50 %).

In terms of seasonality, in this study prevalence of *P. bliccae* was the highest (45.45 %) in the summer and mean intensity reached a peak in winter (11.5 %). The maximum abundance occurred during spring and minimum during autumn with no parasitism. Ünal et al. (2017) reported that *P. bliccae* prevalence was in the highest value in summer.

According to previous studies dealing with other *Paradiplozoon* species, conclusions show that water temperature may affect parasitism. For example, *P. homoion* has been reported in different fish species. Pečínková et al. (2005) found *P. homoion* specimens in *Gobio gobio* with highest prevalence 93.33 % in winter. In Soylu (2007), maximum of monthly prevalence was recorded in January (79.3 %) and lowest in May (43.2 %) from *Pseudophoxinus antalyae* while Koyun and Altunel (2007) reported maximum in summer in *Alburnus alburnus* (69.0 %). Öztürk and Özer (2014) found highest infection percentages in *Scardinius erythrophthalmus* and *Vimba vimba* in summer 54.6 % and in spring 21.1 %, respectively. By Gilbert and Avenant-Oldewage (2016a), infestations of *Paradiplozoon ichthyoxanthon* in *Labeobarbus aeneus* were most intensive (90.0 %) in the summer. Stojanovski et al. (2010) observed differences in invasion of *P. ergensi* according to the season in *Leuciscus cephalus albus* from Lake Ohrid and reported highest value in spring (25.0 %).

There are several studies interested in *Pseudophoxinus* parasites in Turkey. By Soylu and Emre (2007), *Paradiplozoon homoion*, *Dactylogyrus ergensi*, and *Dactylogyrus sphyrna* were reported on the gills of *Pseudophoxinus antalyae* from Kepez I Hydro Electric Power Plant Loading Pond (Antalya, Turkey). Soylu (2007) found that *P. antalyae* were infected by *P. homoion* with an overall prevalence of 54.6 %. Aydoğdu et al. (2011) reported that *Contracaecum* sp. larvae attached in the abdominal cavity of *Pseudophoxinus battalgilae* that lived in Antalya Bay with a total prevalence 47.6 %. Aydoğdu et al. (2014) conducted a study in İncesu Stream – Konya Province and four parasitic helminth species were detected; *Asymphylogora imitans* (28.2 %), *Bothriocephalus acheilognathi* (1.08 %) and *Pomphorhynchus laevis* (52.1 %) in the intestines of *Pseudophoxinus crassus* while *Gyrodactylus latus* (5.4 %) in the gills of host fishes. The study was carried out in Düğer Creek by Innal et al. (2017) and *Lernaea cyprinacea* were found in *P. burduricus* overall prevalence (28.3 %) as mentioned above. Lesley, Aydoğdu, and Emre (2015) dissected *Pseudophoxinus fahrettini* and found 11 specimens of *Pomphorhynchus spindlet truncatus* with 31.4 % prevalence from Aksu, Pazarköy.

Our work, for the first time, shows that the occurrence of *P. bliccae* hosted by *P. burduricus* contributed to the knowledge of distribution of this parasite in Turkey. Additionally, our results have indicated that both seasonal and host factors (age and sex) may influence the levels of infection of *P. burduricus* with this parasite species. Even if there is a need to investigate additional material for species identification and molecular analyses together with a wide geographical investigation to obtain more information about monogenean fauna in the inland waters of Turkey.

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