

Occurrence of *Paradiplozoon* spp. in vimba bream *Vimba vimba* from Lake Köyceğiz, southwest Türkiye, with new data on their distribution

by

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Abstract

Only one species of the genus *Paradiplozoon* (Akmerov 1974) has so far been recorded in *Vimba vimba* (Linnaeus, 1758) from Türkiye. This study was planned to improve the recording of the occurrence of diplozoids in *V. vimba* from a pristine area in a different geographical region. A total of 397 specimens of *V. vimba* were collected from Lake Köyceğiz by commercial fishermen over a period of one year and examined for the presence of diplozoids. Only one species of *Paradiplozoon* was found. Following a detailed morphological examination, the species was identified as *Paradiplozoon bliccae* (Reichenbach-Klinke 1961), which was subsequently confirmed by molecular data. A total of 1025 specimens of *P. bliccae* infected 185 of the 397 *Vimba vimba* specimens examined, with prevalence, mean intensity and abundance of 46.6%, 5.5 ± 4 parasites/fish, 2.5, respectively. The highest prevalence and mean abundance were determined in spring, while mean intensity values were higher in summers. Female hosts showed higher prevalence, mean intensity and mean abundance than males.

This is the first report on the diplozoid *P. bliccae* from the locality and the first molecular characterization of *P. bliccae* from *V. vimba* in Lake Köyceğiz. This study adds to the geographical range of the species.

Key words: diplozoid parasites, seasonal effects, host sex, ITS2 rRNA

1. Introduction

Vimba vimba is a very common fish species distributed in Central Europe, as well as in the basins of the Black Sea and the Sea of Azov. It arrived in Türkiye from Thrace and spread especially in the regions of northern, northwestern and central Anatolia (Geldiay & Balik 1999). Various ichthyo-parasitological studies have been carried out on *V. vimba* from different geographic regions of Türkiye (see, for example, Öztürk et al. 2012; Özer et al. 2013; Öztürk & Özer 2014). According to Özer (2022), 36 different parasite species have been recorded to date in this host species, with helminths having the highest diversity, represented by 29 of the 36 species, the most common of which are monogeneans with 11 species. Of the monogenean species, only two species of the genus *Paradiplozoon* – *Paradiplozoon homoion* (Bychowsky & Nagibina 1959) and *P. bliccae* – have so far been reported from *V. vimba* in Türkiye. It appears from these studies that diplozoid parasite assemblages collected from vimba bream in Turkish inland waters may be depauperate. However, there are no studies on diplozoid parasites of *V. vimba* from Lake Köyceğiz. Therefore, this is the first survey of diplozoids in this host species from this locality.

Moreover, of the previous studies, only two (Unal et al. 2017; İnnal et al. 2020) focused on the frequency of diplozoid parasitic infections or their population dynamics. Therefore, the present study had the following objectives: 1) to provide additional information on the diplozoid parasite fauna of *V. vimba* in Turkish waters; 2) to determine how the prevalence, abundance and intensity of diplozoid infection vary with season and sex of host fish; 3) to expand our knowledge about the geographical distribution and host range of the diplozoid parasite; 4) to provide molecular analysis of the diplozoid parasite collected from the host fish; and 5) to increase the number of reports in GenBank on the molecular characterization of *Paradiplozoon* specimens collected from Türkiye.

2. Materials and methods

Fish sampling in Lake Köyceğiz (Fig. 1), Muğla Province, was carried out seasonally, from October 2019 to July 2020. Lake Köyceğiz (Fig. 1) is a coastal, crenogenic, meromictic wetland located south of the center of the town of Köyceğiz within the borders of Muğla Province in western Anatolia, southern Türkiye. The surface area of the lake is approximately 55 km². Previous limnological studies have established that the



Figure 1

Map of the sampling location.

lake is shaped by karstic cold and hot water sources at the bottom of the lake, which affects the lake's water mass, consisting of hydrochemically different water layers (Anonim 2021). The following streams feed Lake Köyceğiz: Kargıcak, Yangi, Değirmendere, Çamlıdere, Kocaöz, Çakmak, Yuvarlak and Namnam Stream (Buhan 1998; Ertürk 2002; Kazancı et al. 2008).

A total of 397 *Vimba vimba* specimens were caught by commercial fishermen using gillnets. Specimens were placed on a fishing boat in 12-liter cold chain plastic containers containing ice molds and water and immediately transferred to the research laboratory. The fish were then wrapped in aluminum foil to keep them isolated from each other and placed in the freezer. Samples were examined immediately after thawing following standard parasitological methods (Ash & Orihel 1987) to detect diplozoids. Before removing the gills, the sex of each fish was determined based on the location of the gonads in the dissected fish, and the fish was sorted into males (those with testes) and females (those with ovaries). All *Paradiplozoon* specimens were removed from the gills of freshly thawed fish samples under an Olympus stereomicroscope. The number of parasites was recorded and permanent mount slides with a mixture of glycerin and ammonium picrate were prepared (Malmberg 1957; Khotenovsky 1974; Ash & Orihel 1987). For morphometric studies, structures selected for their taxonomic importance and the length of the central hook sickle were measured. All measurements were taken using an ocular micrometer and reported in micrometers unless otherwise stated, with a sample size (n) and a range given in parentheses in the results. The identification of *Paradiplozoon* specimens was

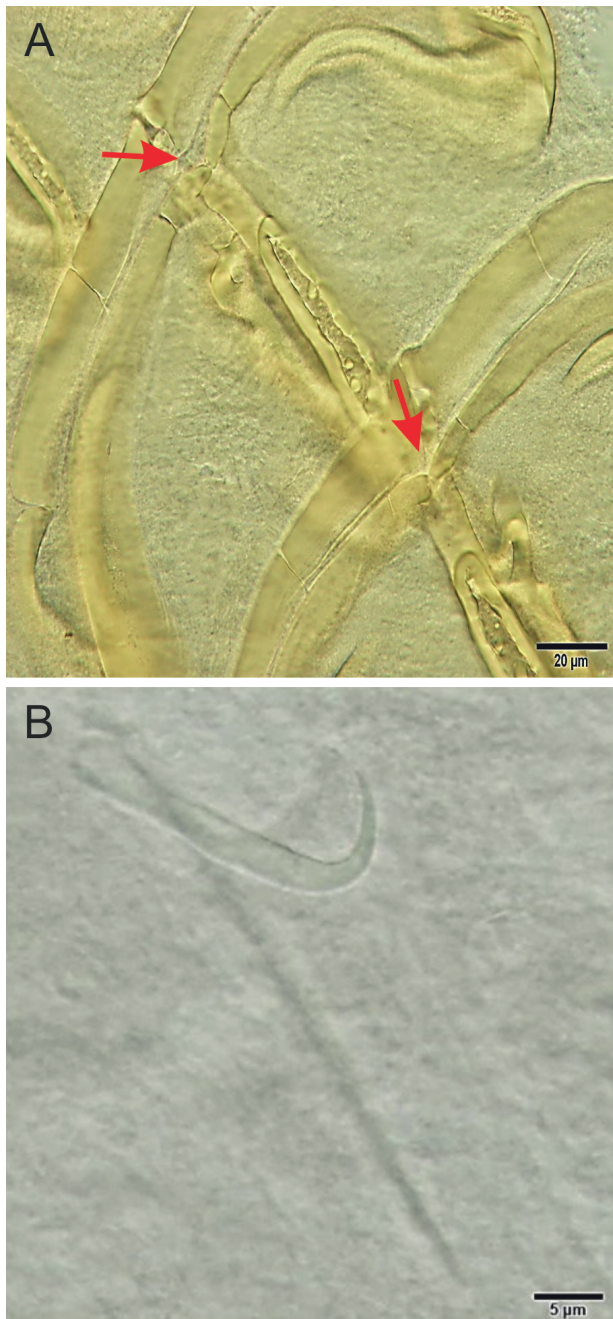


Figure 2

Clamp of *Paradiplozoon bliccae* isolated from *Vimba vimba* in Türkiye; A – posterior view; B – central hooks showing the handle.

carried out according to diagnostic morphological keys of Gussev (1985), Bykhovskaya – Pavlovskaya (1962), Khotenovskiy (1974) using an optical microscope. The morphological species identification of diplozoids was supported by molecular analysis of ITS2 rRNA sequences performed on the same parasite specimens (n = 20). In this regard, the collection of diplozoid

samples for molecular identification in this study was carried out following the method described by Aydogdu et al. (2020a,b). Twenty diplozoid specimens/PCR products were delivered to the Laboratory of Parasitology, Department of Public Health and Infection Diseases of “MS LAB (Eskisehir, Türkiye)” for molecular analysis. The sequence data from the 5.8S rRNA gene (ITS 2) of diplozoid parasites provided in this laboratory were published in GenBank (Genbank accession number: OQ435904, data not shown). The acquired nucleotide sequences were then compared to *Paradiplozoon* spp. sequences from *Squalius fellowesii* (Günther, 1868) and other related species of *Paradiplozoon* spp. isolates from the GenBank database.

For this purpose, the BLAST (www.ncbi.nlm.nih.gov/BLAST/) algorithm (Altschul et al. 1980) was used for a homology search of 5.8S rRNA gene (ITS 2) sequences. The phylogram analysis (Fig. 3) used the Maximum Likelihood Tree method in Mega 10 software (Kumar et al. 2018).

Photomicrographs of all parasite specimens were taken using a camera mounted on a Leica DMR microscope with phase contrast and an Olympus BX-50 research microscope.

For statistical evaluation, data on *Paradiplozoon* specimens were categorized according to season and host sex. Prevalence, mean intensity and mean

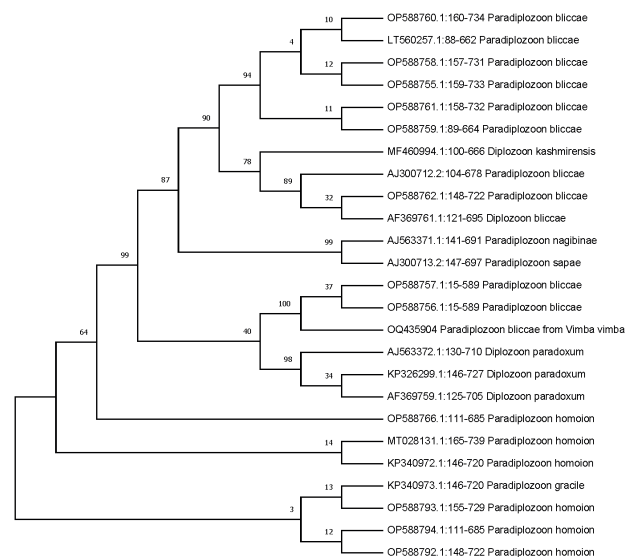


Figure 3

Phylogenetic tree based on Maximum Likelihood Analysis in some *Paradiplozoon* spp. isolates from the GenBank database and the present study. Bootstrap support values lower than 90% are not shown.



abundance of infection were calculated following Bush et al. (1997). Standard deviation was calculated using Microsoft Excel (Office 2000). All parasitological data (seasons and host fish sex difference) were analyzed using the SPSS v. 28 software package for Windows. A significance level of $p \leq 0.05$ was used.

3. Results

Of the 397 *Vimba vimba* individuals examined, only one monogenean species was found on the gills. Based on their morphological characteristics, e.g. including the shape of the trapezoidal spurs and anterior joining sclerites of the clamps, the parasites were identified as *Paradiplozoon* (Fig. 2). The shape of the anterior joining sclerites in the specimens examined is typical, forming a figure in the shape of “_|_” (indicated by the arrow in Figure 2A). The size of the central hook sickle (Fig. 2B) ranged from 22.5 to 25 (23.5; $n = 10$) μm . The identification of *Paradiplozoon bliccae* was corroborated by the trapezoidal spurs, the anterior joining sclerites of the clamps and the size of the central hook sickle. ITS2 was amplified from the specimens ($n = 20$) and the length of the PCR product was approximately 575 bp. The sequence data were published in GenBank (Genbank accession number OQ435904, <http://www.ncbi.nlm.nih.gov>, data not shown). The BLAST analysis of diplozoid specimens obtained in the current study showed similarity with the *P. bliccae* samples from other studies (OP588761, OP588755, OP588760, OP588758, OP588757 and OP588756; Nejat et al. 2023) and those presented by Ünal et al. (2007) with accession number LT560257 confirming the identification of the species.

The most suitable phylogenetic tree model for the sequences was determined to be the Tamura-Nei+Gamma distribution (+G) by MEGA 11 software. A phylogenetic tree was created using the Maximum Likelihood statistical method and is shown in Figure 3.

A total of 1025 specimens of *Paradiplozoon bliccae* were found in 185 of the 397 fish examined (with prevalence, mean intensity and abundance of 46.6%, 5.5 ± 4 parasites/fish, 2.5, respectively). The highest prevalence was recorded in spring – 86.3% (Table 1). The highest mean abundance was also observed in spring, while mean intensity was higher in summer (Table 1). The species was not detected in autumn. Statistically significant differences were found in the number of parasites collected per season (Kruskal–Wallis H test, $p < 0.01$).

The infection per host sex is shown in Table 1. In females, the prevalence is higher than in males. The mean intensity is also higher in females (Table 1). Although females were more infected, this was not statistically significant based on Mann–Whitney U test ($p = 0.430$).

4. Discussion

Although many ichthyo-parasitological studies have been carried out on *Vimba vimba* from different geographic regions, the diplozoid parasite fauna is unfortunately still poorly studied. This study investigated the occurrence of diplozoid parasites in *V. vimba* from Lake Köyceğiz. Only one diplozoid species was identified, i.e. *Paradiplozoon bliccae*. This is the first report on the diplozoid *P. bliccae* from host fish in this locality. The present study increases the number of localities of diplozoid species recorded in *V. vimba* in Turkish inland waters to three. As for the identifications of the diplozoid parasite, we described it based on the morphological characteristics, namely the typical shape of anterior joining sclerites forming a figure in the shape of “_|_” (indicated by the arrow in Figure 2A) and the size of the central hook sickle (Fig. 2B). We also confirmed the identification of the species as *P. bliccae* using molecular data. The sequence data were 100% consistent with the sequence of *P. bliccae* from Türkiye, submitted on 4 October 2022 by Nejat et al.

Table 1

Prevalence and intensity values of *Paradiplozoon bliccae* in *Vimba vimba* from Lake Köyceğiz according to season and host sex.

		Infection parameters			
		Prevalence (%)	Mean intensity \pm SD	Abundance	Total parasite no
Seasons	Autumn ($n = 31$)	This species was not detected			
	Winter ($n = 69$)	53.6	3.1 ± 1.9	1.6	117
	Spring ($n = 73$)	86.3	5.8 ± 3.7	5	368
	Summer ($n = 224$)	37.9	6.3 ± 4.6	2	540
Fish sex	Female ($n = 269$)	48.3	5.5 ± 4	2.6	724
	Male ($n = 128$)	42.9	5.4 ± 4.1	2.3	301

(2023) with accession numbers OP588761, OP588760, OP588758, OP588757, OP588755 in GenBank and had a 0.17% nucleotide difference with *P. bliccae* presented by Unal et al. (2017) with accession number LT560257, deposited in the GenBank database. These findings increase the number of reports on diplozooids and their molecular characterization.

Although *P. bliccae* shows a wide host range in the Middle East (Nejat et al. 2023), it is only found in Cyprinidae hosts. The species was first reported from the inland waters of Türkiye, i.e. Doğanbaba Creek (Unal et al. 2017). Since then, the parasite has been reported from the following fish species in Turkey: *Pseudophoxinus burduricus* (İnnal et al. 2020), *Squalius fellowesii*, *Vimba mirabilis*, *Vimba vimba*, *Ladigesocypris ghigii*, *Petroleuciscus ninae*, *Luciobarbus kottelati*, *Barbus xanthos* and *Capoeta aydinensis* (Nejat et al. 2023). The above-mentioned fish species are distributed in six different habitats in Türkiye. With this study, the number of habitats where *P. bliccae* has been recorded in Türkiye increased to seven.

Other *Paradiplozoon* (except for one parasite not identified to species level) occurring in Türkiye include *Paradiplozoon homoion* and *P. barbi* (Reichenbach-Klinke 1951), *P. bingolensi* (Civanova et al. 2013), *P. megan* (Bychowsky & Nagibina 1959), *P. pavlovskii* (Bychowsky & Nagibina 1959) (Özer 2021) and *P. koubkovae* Rehulkova, Nejat and Benovic (Nejat et al. 2023). Furthermore, this species is found in *Blicca bjoerkna*, *Abramis brama*, *Scardinius erythrophthalmus*, *Vimba vimba*, *V.v. persa* and *V.v. tenella* in rivers flowing into the Baltic, Black and Caspian seas (Pugachev et al. 2009; Nejat et al. 2023).

As for the infection rates of this diplozoid species in different fish species in Türkiye, Unal et al. (2017) and İnnal et al. (2020) reported lower infection in their studies: prevalence and mean intensity values were 23.3% and 6.1 parasites/fish (however, this value is higher than our value) in *Pseudophoxinus burduricus*, and 30.9% and 3 parasites/fish in *Squalius fellowesii* from Doğanbaba Creek, respectively. These findings are inconsistent and lower than in our report.

As for the seasonal variation in *P. bliccae* infection, it has been reported in two fish species in Türkiye (Unal et al. 2017; İnnal et al. 2020). Unal et al. (2017) recorded the highest infection in the Aegean chub, *S. fellowesii*, and İnnal et al. (2020) in *P. burduricus*, both in summer. In this study, the highest infection was recorded in spring. In addition, the mean intensity values recorded by these authors are also inconsistent with our study. Koyun (2001), Öztürk (2005), Soylu (2007), Unal et al. (2017), Aydogdu et al. (2020a,b) and İnnal et al. (2020) studied the seasonal variation of various *Paradiplozoon* spp. in different fish species in Türkiye. Some authors (Aydogdu et al. 2020a,b; Soylu 2007) reported the

highest prevalence in winter, while others (Koyun 2001; Öztürk 2005) reported a higher prevalence in summer. The above authors suggested that this may be due to a combination of differences in parasite species, different rates of parasite development in different localities, hosts and environmental conditions. We support their suggestion.

In our study, the prevalence, mean intensity and abundance of *P. bliccae* were higher in females than in males ($p = 0.430$). İnnal et al. (2020) also reported the highest prevalence in females of *P. burduricus*. Unal et al. (2017), on the other hand, reported that it was higher in males of *S. fellowesii*. The results of the present study corroborate those of İnnal et al. (2020).

5. Conclusions

This study increases the geographical distribution of *P. bliccae* and the number of definitive hosts of *P. bliccae* recorded in Türkiye. In addition, the present study adds some valuable information on seasonal occurrence of *P. bliccae* and its physical parameter (sex). Furthermore, the present study increases the number of records in GenBank for *Paradiplozoon* specimens from Türkiye, and studies on more locations and more genes are needed to better understand the genomic features of *P. bliccae*. This study highlights the need for more ichthyo-parasitological surveys in previously understudied localities.

Declarations

Data Availability Statement

No data availability statement.

Compliance with ethical standards

No ethical approval was required in Türkiye as this study did not involve clinical trials or experimental procedures. No treatments or experiments were performed on live animals during the study. All sampling and laboratory work on the fish complied with the animal welfare regulations of the Ministry of Agriculture and Forestry of the Republic of Türkiye.

Conflict of interest

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



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