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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Investigations on *Paradiplozoon bliccae* (Reichenbach-Klinke, 1961) (Monogenea: Diplozoidae) found in *Capoeta aydinensis*, an endemic fish in Türkiye, based on ecological, molecular and host related factor approaches

Türkiye'nin endemik balıklarından *Capoeta aydinensis*' ten *Paradiplozoon bliccae* (Reichenbach-Klinke, 1961) (Monogenea: Diplozoidae)'nin ekolojik, moleküler ve konak ilişkili faktör yaklaşımıyla araştırmalar

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Abstract: The present study investigated diplozoid parasites in an endemic species, *Capoeta aydinensis* Turan, Küçük, Kaya, Güçlü & Bektaş, 2017 in Köyceğiz Lake, near Muğla province (Türkiye). The aim of this research is to improve a record of diplozoid species occurrence in *C. aydinensis*, an endemic fish species by collecting data from a previously unexplored locality situated in a different geographical region of Türkiye. A total of 187 individuals of *C. aydinensis* were collected by using fishing nets from October 2019 to July 2020 and examined for the presence of diplozoid species. Only one species of diplozoid has been recorded, known as *Paradiplozoon bliccae* (Reichenbach-Klinke, 1961), which has been identified morphologically and confirmed through molecular analysis. The nucleotide sequences of the parasite's nuclear internal transcribed spacer (ITS2) gene marker were determined as well as phylogenetic analyses by using Bayesian inference (BI) analyses. On the basis of the molecular findings, the morphological identification of the diplozoid parasite species was confirmed. Of 187 fish sampled, 27 were infected with 117 *P. bliccae*, representing an abundance of 0.6, a mean intensity of 4.3 and a prevalence of 14.4%. The prevalence and mean intensity of infection were based on the season and sex of the host. The highest values of infection for prevalence, mean intensity and abundance were found in summer. Meanwhile, mean intensity and abundance of *P. bliccae* were higher in males, the prevalence was higher in females. To our knowledge, the present study is the first ichthyoparasitological study of *C. aydinensis* in Köyceğiz lake, near the province of Muğla in Türkiye. Furthermore, sequence data of *P. bliccae* from fish hosts in this locality were reported to GenBank for the first time as part of this study. Therefore, this study widens the host range of this parasite species in Türkiye.

Keywords: Köyceğiz lake- *Capoeta aydinensis*, *Paradiplozoon bliccae*, molecular approach, seasonal effects, host sex

Öz: Bu çalışma, Muğla (Türkiye) ili yakınlarındaki Köyceğiz Gölü' nde bulunan endemik *Capoeta aydinensis* Turan, Küçük, Kaya, Güçlü & Bektaş, 2017'in diplozoid parazitleri üzerine yapılmıştır. Araştırmanın amacı, Türkiye' nin farklı bir coğrafi bölgesinde daha önce keşfedilmemiş bir lokasyondaki endemik balık türü *C. aydinensis'* in diplozoid türlerinin varlığına ilişkin kayıtların geliştirilmesidir. Ekim 2019 – Temmuz 2020 tarihleri arasında araştırma alanından balık ağları kullanılarak toplam 187 *C. aydinensis* bireyi toplanmış ve diplozoid türlerinin varlığı açısından incelenmiştir. Ayrıntılı morfolojik tanımlamanın ardından yalnızca bir diplozoid türü, *Paradiplozoon bliccae* (Reichenbach-Klinke, 1961) tanımlanmıştır. Bu morfolojik tür tanımlamasını moleküler yöntemlerle doğrulamak için, konak balıktan alınan parazitin internal transcribed spacer 2 (ITS2)gen bölgelerinin nükleotid dizileri belirlenmiş ve Bayesian Inference (BI) algoritmaları kullanılarak filogenetik analizleri yapılmıştır. Elde edilen moleküler bulgulara göre diplozoid parazit türünün morfolojik olarak tanımlanması doğrulanmıştır. Diplozoid parazit dizileri ayrıca LT560257 erişim numarasıyla GenBank' ta saklanan *Paradiplozoon bliccae* dizileriyle de yüksek homoloji (%99.32) göstermiştir. İncelenen 187 balıktan 27'sinin toplam 117 *P. bliccae* ile 14.4%; 4.3; 0.6 sırasıyla enfeksiyon oranı, enfekte balık başına ortalama parazit sayısı ve incelenen balık başına ortalama parazit sayısı enfekte olduğu belirlenmiştir. İlave olarak, mevsime ve konakçı cinsiyetine göre enfeksiyon oranı ve enfekte balık başına ortalama parazit sayısı hesaplanmıştır. Enfeksiyon oranı, enfekte balık başına ortalama parazit sayısı ve incelenen balık başına ortalama parazit sayısı için en yüksek değerler yaz aylarında bulunmuştur. *P. bliccae*' nin enfekte balık başına ortalama parazit sayısı ve incelenen balık başına ortalama parazit sayısı erkeklerde daha yüksekken, enfeksiyon oranı dişilerde daha yüksek bulunmuştur. Bildiğimiz kadarıyla bu çalışma, Muğla ili Köyceğiz Gölü' nde *C. aydinensis*' in ilk ihtiyoparazitolojik araştırmasıdır. Ayrıca bu çalışma ile ilk kez bu lokalitedeki konak balıklardan elde edilen *P. bliccae*' nin sekans dizi verileri de GenBank'a raporlanmıştır. Böylece bu çalışma, bu parazit türünün Türkiye'deki konak yelpazesini üçe, yer sayısını ise ikiye çıkarmıştır.

Anahtar kelimeler: Köyceğiz Gölü, *Capoeta aydinensis*, *Paradiplozoon bliccae*, moleküler yaklaşım, mevsimsel etki, konak cinsiyeti

INTRODUCTION

According to Froese and Pauly (2022), 409 species of freshwater fish have been reported from Türkiye's inland waters so far. Endemic fish species represent 194 of these species (Çiçek et al., 2018). One of these endemic species,

Capoeta aydınensis Turan, Küçük, Kaya, Güçlü & Bektaş, 2017 is distributed in clear and moderately flowing waters with a substrate of stones and pebbles (Froese and Pauly, 2022). It occurs in the Büyük Menderes River as well as Dalaman, Namnam and Tersakan in the freshwater ecosystems (Froese and Pauly, 2022). Despite the increase in the number of studies on helminth parasites of endemic fish species in Türkiye in recent years, it is evident from current literature that comprehensive studies on the helminth parasites of endemic fish species are needed due to the determination of the helminth fauna of most endemic fish species distributed in Türkiye. In confirmation of the above information, there is only one ichthyo-helminthological record for *C. aydınensis* so far in Türkiye (Nejat et al., 2023). In addition to these, Diplozoid parasite species also appear to be very poorly represented in Türkiye fauna. Besides these studies, Özer (2021) found that the genus *Paradiplozoon* Akhmerov, 1974 is represented in Türkiye by six previously known species of parasites and one unspecies identified parasite plus that no previous study has found *Paradiplozoon* spp in this host fish in the locality. Moreover, *Paradiplozoon bliccae* has been recorded in only three studies in Türkiye so far (İnnal et al., 2020; Unal et.al., 2017; Nejat et.al., 2023). Diplozoids are a unique family of Monogenea that are common and widespread gill parasites of cyprinid fishes (Pecínková et. al., 2005).The frequency diplozoid parasite detections in host fish is not constant for all species, as is the case for other helminth parasites (Yunchis, 1988), and varies depending on season, host biology, host age, size, and salinity and temperature of the water of the location in the study area.

Therefore, the current study aims to: (i) Determine the *Paradiplozoon* species that infect *C. aydinensis* by collecting data from a previously unexplored location in a geographic region in Türkiye, which is different from the previous records. (ii) Increase the number of members within the Diplozoidae family documented in Türkiye. (iii) Expand our understanding of the geographical distribution and host range of Diplozoid parasite specimens in Türkiye's waters. (iv) Determine whether the influence of season and host sex on the occurrence of these parasites in *C. aydinensis*.

MATERIALS AND METHODS

Sampling and parasitological analysis

A total of 187 individuals of *Capoeta aydinensis* were caught by commercial fishermen in Köyceğiz lake, located in Muğla province. The collection was seasonal, from October 2019 to July 2020. The fish samples were placed in 12-liter cold chain plastic containers filled with ice molds on a fishing boat and were immediately transferred to the research laboratory. Then the fish were wrapped in aluminum foil to keep them separate and were placed in the freezer. On examination day, the fish were defrosted (in tap water) for approximately 20 minutes at room temperature. The remaining fish were defrosted one by one, following the completion of the previous examination. The sex of each fish was determined during dissection, and then the gills were examined for *Paradiplozoon* specimens from newly defrosted fish individuals by using an Olympus stereomicroscope. The number of parasites was counted and preserved with a mixture of glycerin–ammonium picrate (Malmberg, 1957; Khotenovsky, 1974). The identification of *Paradiplozoon* specimens were performed according to morphological keys of Gussev (1985); Bychowskaya–Pavlovskaya (1962); Khotenovsky (1985) and other available references with an optical microscope. Finally, the collected diplozoid species were fixed and stored in absolute ethanol at +4 C^0 until DNA isolation and delivered to the Laboratory of Parasitology in Department of Public Health and Infection Diseases of "MS LAB (Eskişehir, Türkiye)", for molecular identification. The photomicrographs of all parasite specimens were taken by using a photographic camera mounted Leica DMR microscope with phase contrast and an Olympus BX-50 research microscope.

Molecular Analyses

Before DNA isolation, the samples fixed in alcohol were first centrifuged at 3000 RPM in 1.5 ml microcentrifuge tubes, noting that ethanol in the upper part was removed. The Genomic DNA of the parasites were extracted using the A.B.T.™ DNA Purification Kit (Qiagen, Hilden, Türkiye), by following the standard manufacturer-recommended protocol. The purity levels of the isolated DNA samples were measured first with the Maestrogen Nano (Maestrogen, Taiwan) spectrophotometer and then with the Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) systems. After isolation, DNA samples were amplified using Promega goTaq polymerase and buffers. A fragment of ITS2 rDNA was amplified by using primers:

D (5'GGCTYRYGGNGTCGATGAAGAACGCAG-3') and

B1 (5'GCCGGATCCGAATCCTGGTTAGTTTCTTTTCCT-3').

Polymerase chain reaction (PCR) was carried out according to the reaction conditions of Matejusová et al. (2001). PCR amplicons were sent to MS LAB (Eskisehir, Türkiye) for Sanger sequencing (Applied Biosystems, Forster City, CA). Post-PCR samples were verified using the Qiagen QIAxcel Advanced capillary electrophoresis system (Qiagen, Hilden, Germany) to 293 base pairs. The resulting sequencing was analyzed to distinguish species using NCBI blast program (Altschul et al.,1990). Sequence data was published to GenBank (GenBank accession number: OP723498, http://www.ncbi.nlm.nih.gov). The acquired nucleotide sequences were compared to *Paradiplozoon* spp. sequences supplied and found to be more than 95% identical in GenBank. A phylogram was generated using the MrBayes 3.1.2 (Huelsenbeck et al., 2001) program.

For phylogenetic analyses, the sequence obtained in this study was aligned with the data in the literature using the ClustalW algorithm in the MEGA-X (Kumar et al., 2018) program. As a result of the alignment, non-informative parts of the sequences were cut at the start and at the end. Then, the Modeltest v. 2.1.5 (Darriba et al., 2012) program was used to determine the mutation model that best matched the data

obtained. Among a total of 56 mutation models, it was determined that the most appropriate mutation model for the data set was the GTR model (general time reversible model) according to the Akaike information criterion (AIC). MrBayes 3.1.2 (Huelsenbeck et al., 2001) program was used to construct the phylogenetic tree. As an outgroup, *Sindiplozoon* spp. was used. Markov Chain Monte Carlo analysis was carried out over 4 chains. In addition, analyses were carried out for 10 million generations until the split frequency fell below 0.01. When the analysis reached saturation (ESS values), it was monitored using the Tracer v.1.7 (Rambaut et al., 2018) program. 25% of the trees obtained by sampling every 1000 generations were removed by burn-in. The consensus tree was obtained as a result of the analysis that was viewed and edited in the Figtree v1.4.2 (Rambaut 2014) program (Figure 2).

Statistical analysis

Data on *Paradiplozoon* species was categorized according to the seasons and the sex of the host fish. The levels of prevalence, mean intensity and abundance of infection as defined by Bush et al. (1997) were calculated.

Standard statistical computations (standard deviation) were carried out using Microsoft Excel (Office 2000). Kruskal-Wallis (more than two groups) tests were applied to find significant differences in the mean intensity of the parasite species for the size and seasons of host fish. The Mann-Whitney U test (two groups) was used to determine the correlation between the intensity of each helminth species infection and the host sex. The significance level of $\alpha \leq 0.05$ was used. All statistics analyses were performed using SPSS v. 28 for Windows.

RESULTS

On the gills of 187 *Capoeta aydınensis* individuals, one species of diplozoid was identified. Based on our detailed studies on haptor and attachment apparatures morphology of the species, *Paradiplozoon bliccae* was identified (Figure 1A-B), and confirmed by molecular analysis. A total of 117 specimens of this parasite were found infecting 27 of the 187 fish examined, with prevalence (14.4%), a mean intensity (4.3 parasite/fish) and abundance (0.6). The seasonal variation of the infection of *P. bliccae* was also investigated in our study.

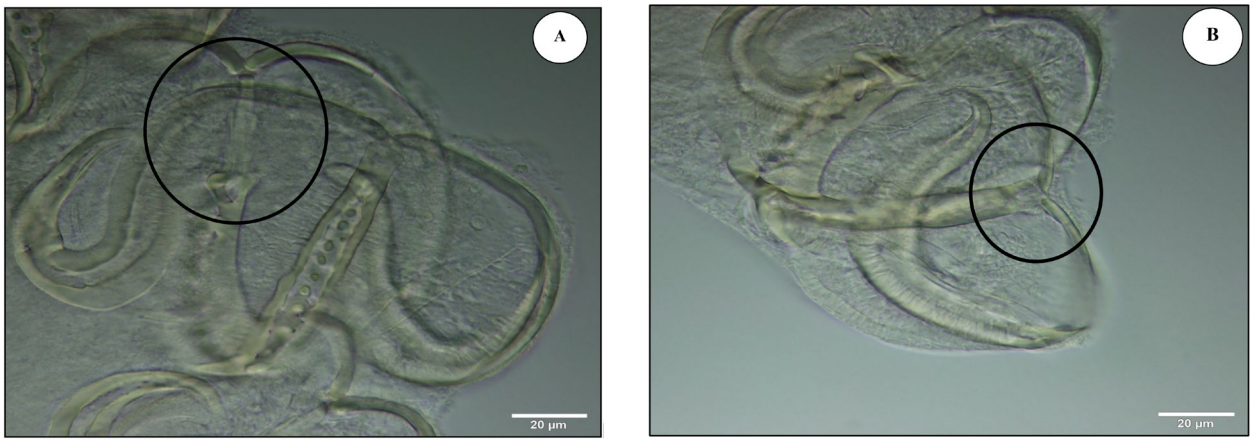

Figure 1. A clamp of *Paradiplozoon bliccae* isolated from *Capoeta aydinensis*; **A**-posterior view. - posterior end of median sclerite (circle indicates); **B**-anterior view. - anterior end of median sclerite. Detail of the trapeze spur and anterior joining sclerites forming a specific T-shape of the clamps (circle indicates)

Changes in infection parameters in relation with seasons are shown in Table 1. Seasonal prevalence, mean intensity and abundance of this parasite species differed between seasons. During spring, a total of 20 specimens of *P. bliccae* were found in 11 of 57 fish examined, yielding a prevalence of 19.2%, a mean intensity of 1.8 parasites/fish, and an abundance of 0.3. For summer, 58 fish were caught and a total of 97 parasites were found in 16 of the 58 fish examined (prevalence 27.5%, mean intensity 6 parasite/fish and abundance 1.6.

Table 1. Prevalence and intensity values of *Paradiplozoon bliccae* in *Capoeta aydinensis* from Köyceğiz Lake according to seasons and host sex

		Infection parameters			
		Prevalence (%)	**Mean intensity ± SD**	**Abundance**	**Total parasite no**
Seasons	Autumn (n=16)	-	-	-	-
	Winter (n=56)	-	-	-	-
	Spring (n=57)	19.2	1.8±1.07	0.3	20
	Summer (n=58)	27.5	6±3.15	1.6	97
Fish sex	Female (n=123)	16.2	3.3±2.36	0.5	66
	Male (n=60)	11.6	7.2±3.86	0.8	51

This species was not detected in the autumn and winter samples. Seasonal prevalence of infection was higher in summer, 27.5%, the highest mean intensity and abundance values were also found in summer (Table 1). According to Kruskal Wallis's-H test, there were statistically significant differences in number of parasites collected per season ($p<0.001$).

A total of 187 *C. aydinensis* individuals (123 females, 60 males, and 4 not identified) were examined for diplozoid parasites. *P. bliccae* was found in 7 of 60 male fish examined, with prevalence, mean intensity and abundance of infection of 11.6%, 7.2 parasite/fish and 0.8, respectively. A total of 66 specimens of this parasite species infected 20 of the 123 female examined fish (Table 1).

The prevalence of *P. bliccae* was higher in females (16.2%) when compared to males (11.6%) while the mean intensity and abundance of this species was higher in males (Table 1). There was a statistically significant sex–related difference in the number of this species based on Mann–Whitney U test ($p=0.02$). The partial sequence of 293 nucleotides of the present diplozoid species was obtained in the present study and is represented in Figure 2. The BLAST analysis of these sequences revealed high homology (99.32%) and (99.32%) with *Paradiplozoon bliccae* sequences deposited by Unal et al. (2017) (GenBank LT560257), Matějusová et al. (2001) (GenBank AJ300712), respectively and high similarity (98.98%) with *P. bliccae* collected from *Squalius cii* published by Sicard et al., (2001) (GenBank AF369761) (Figure 2). The data sequence of this present diplozoid species was deposited in GenBank (accession number: OP723498). Multiple alignments were performed on *Paradiplozoon* specimens using sequencing data from NCBI GenBank and a phylogenetic tree was generated (Figure 2).

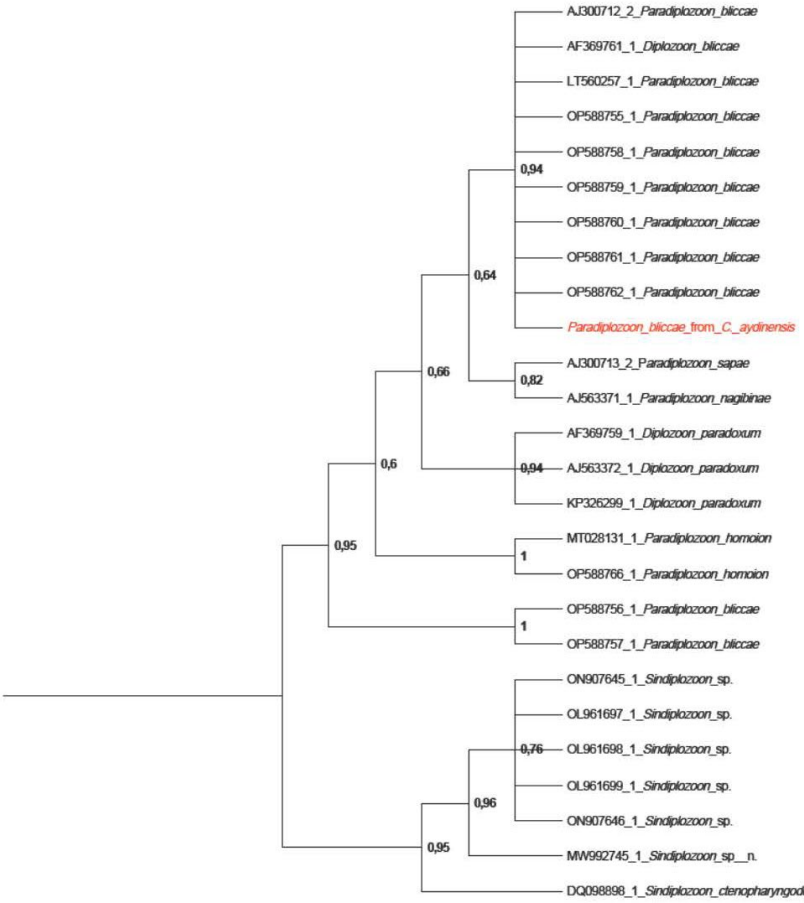

Figure 2. Phylogenetic tree for the relationships of *Paradiplozoon bliccae Paradiplozoon* spp., sequences supplied and found to be more than 95% identical in GenBank. Scale bar (=2) represents the expected number of substitutions per nucleotide

DISCUSSION

Capoeta aydinensis is one of the endemic fish species that spread in the Büyük Menderes river drainages and Dalaman, Namnam and Tersakan in South-Western Türkiye (Turan et al., 2017). To our knowledge, there is only a single ichthyohelmintological data reported for *C. aydınensis* in Türkiye so far (Nejat et al., 2023).

In this study, the diplozoid parasitesfauna was investigated in *C. aydınensis* from Köyceğiz Lake from Southwestern Anatolia, Türkiye. Only one diplozoid species was identified, namely *Paradiplozoon bliccae*. It was described based on the haptor and the attachments apparatus morphology. The identifying feature of *P. bliccae* is the typical shape of anterior

joining sclerites forming a specific T-shape and its trapeze spurs shapes, anterior joining of sclerites of the clamps and the size of the central hook sickle were distinguished it from other closely related species belonging to the genus *Paradiplozoon*. (Figure 1A-B, circle indicates). In addition to the morphological characters used to identify species belonging to the genus *Paradiplozon* above, to our best knowledge, in identifying the morphological types of monogenic parasites belonging to the Diplozoidae family, the following characteristics are used; measurements and shapes of the clamps and central hook, anterior/posterior part ratios of the body, shape of the intestinal cecum ending at the back, etc. Structures selected according to taxonomic importance are used. Situations such as the continuous growth of these structures, which are important in species identification, and the change in metric measurements even within the species, make species identification of diplozoid parasites very difficult. Therefore, it is clear that it should be confirmed using molecular analysis as well as morphological characters. For these reasons, molecular analyses were carried out. And in our study, 293 bp of ITS2 gene region was replicated and compared with the data in GenBank. The molecular identification and DNA sequences using the ITS2 gene showed that the specimens from *C. aydinensis* in Köyceğiz Lake were identified as *Paradiplozoon bliccae* (Figure 2). Our samples were clustered into *Paradiplozoon* and into *Paradiplozoon bliccae* species in the BI phylogenetic tree (98- 99). For example, the sequences of *P. homoion* are 100% similar to accession number MT417728 sequences presented by Benovics et. al., (2020). Several previous reports have indicated that in support of morphological data (Unal et. al., 2017; İnnal et al., 2020; Nejat et al., 2023), they have used the ITS2 gene 5.8S rRNA gene to confirm the morphological identification of these diplozoid parasites.

In this aspect, the present study raises the number of reports on the molecular characterization of *Paradiplozoon* specimens collected from Türkiye. In addition, sequence data of *P. bliccae* from host fish in this locality were reported to GenBank for the first time with this study. In addition, this is the first survey on the ichthyo-helmintological data for this host fish in Köyceğiz Lake in Türkiye. Therefore, this study expands localities for *P. bliccae* in Türkiye.

In the present study, a total of 117 specimens of *P. bliccae* were found in 27 of 187 *C. aydınensis* examined with prevalence and mean intensity of 14.4% and 4.3 parasite/fish respectively. *P. bliccae* has been previously reported from only two fish species living in different habitats in Türkiye: *Pseudophoxinus burduricus* Küçük, Gülle, Güçlü, Çiftçi and Erdoğan, 2013 *(*Teleostei: Cyprinidae) and *Squalius fellowesii* Gunther, 1868 (Teleostei: Cypriniformes) both collected from Doğanbaba Creek with prevalence and mean intensity varying from 23.3% and 6.1 parasite/fish to 30.9% and 3 parasite/fish respectively (Unal et al., 2017; İnnal et al., 2020). These findings are inconsistent with ours. Considering the above records, this present study adds new data to the infection parameters of this diplozoid species. Furthermore, *Blicca bjoerkna* Linnaeus, 1758 (Cyprinidae) has been designated as a main host for this parasite, despite the fact that this diplozoid species has also been recorded in different studies from various freshwater fish species in Europe and Asia (Matějusová et al., 2001; Al-Nasiri, 2009; Sobecka et al., 2014). As for the infection results of this species in their study: infection prevalence value was 13.7%, 18.2% in *Cyprinion macrostomum*, *Cyprinus carpio* respectively from Tigris River (Al-Nasiri, 2009) 1.8% *Leuciscus idus* from Dabie Lake. Moreover, as far as we know up to now, different diplozoid specimens especially, *P. homoion* have been also recorded in previous studies in Türkiye (see, for example, Koyun, 2001; Öztürk, 2005; Soylu and Emre, 2007; Aydoğdu et al., 2020a,b). In the studies conducted on different fish species distributed in different localities in Türkiye, the prevalence levels of infection of parasite specimens belonging to the genus *Paradiplozoon* varied between 1.3% and 73.6%. However, as the authors, we suggest that the formation and distribution of diplozoid parasite specimens recorded above may be due to a combination of differences in biotic and abiotic ecological characteristics variables of the geographical location. While similarities in the prevalence level of diplozoid specimens in fish living in the same habitat can be explained by similarities in biotic and abiotic factors observed in the same locality. The differences can be attributed to the diversity of biotic and abiotic factors that vary from one aquatic ecosystem to the next.

The seasonal variation of the infection of *P. bliccae* was also investigated in our study. The prevalence and mean intensity levels of this species were both higher in summer (27.5%; 6 parasite/fish respectively) (Table 1). Seasonal variation of *P. bliccae* infection rates has also been studied in different fish species in Türkiye so far (Unal et al., 2017; İnnal et al., 2020). Seasonal variation in infection rates of Aegean chub (*Squalius fellowesii*) by Unal et al., 2017) and Burdur spring minnow (*Pseudophoxinus burduricus*) by İnnal et al., 2020) from Doğanbaba Creek, Burdur have studied. They recorded the highest infection prevalence value of *P. bliccae* in the summer (59% in *S. fellowesii*, 45.4% in *P. burduricus*). However, in both studies, the researchers recorded the highest intensity values of this species in different seasons. Unal et al. (2017) found the highest mean intensity values of this species in autumn samples (3.7 parasite/fish) while İnnal et al. (2020) recorded the highest intensity value of this species in winter samples (11.5 parasite/fish). The season with the highest infection prevalence values for this species recorded in this study was consistent with the above studies, but the season with the highest mean intensity values recorded is inconsistent.

The authors (Koyun, 2001; Öztürk, 2005; Soylu and Emre, 2007; Unal et al., 2017; Aydogdu et al., 2020a,b; İnnal et al., 2020) studied the seasonal variability of *Paradiplozoon* spp. infection in different fish species in Türkiye and suggested that this might be due to a combination of differences in the parasite species, different rates of parasite development in different waters, the host and environmental conditions. We also support their suggestion.

In the present study, the mean intensity and abundance of *P. bliccae* infection were higher in male than female hosts (Table 1). However, the prevalence of this species was higher in females (16.2%) compared to males (11.6%). İnnal et al. (2020) recorded the highest prevalence levels of infection in female individuals of *P. burduricus*. The results of the present study also confirm the findings of İnnal et al., (2020). In contrast to our study, Unal et al., (2017) found the highest prevalence levels of this species in male fish in *S. fellowesii.* According to Rohde (1978) and Kennedy (1972), the parasites may infect both sexes differently. They suggested that this might be resulted to differences in colour, hormonal features, mucus, stress, the different feeding habits and habitat used between sexes. In our study, the host sex had a significant influence on *P. bliccae* infection parameters. The differences in these findings may be the result of the above-mentioned factors varying between sexes. This information may indicate that the female individuals of *P. burduricus* and *C. aydinensis* have similar behaviour or feeding habits.

CONCLUSION

In this study, a total of 187 individuals of endemic fish species were examined for diplozoid parasites from October 2019 to July 2020 in Köyceğiz Lake from Southwestern Anatolia, Türkiye. By using morphological and anatomic assessment, only one diplozoid species, *Paradiplozoon bliccae*, was identified, it was confirmed by molecular characterization that these specimens recorded in the host fish are indeed *P. bliccae*. To the best of our knowledge, this is the second ichthyoparasitological study for *C. aydinensis* in Türkiye as well as the first record of *P. bliccae* from this previously unexplored host and region. It is, therefore, a new locality for the distribution of *P. bliccae*. Furthermore, this study adds new valuable information to the molecular characterization of this species collected from Türkiye. In addition, sequence data of *P. bliccae* from host fish was reported to GenBank for the first time from Köyceğiz Lake with this study. Additionally, this study provides further insight into how the infection parameters of this species vary with seasons and host fish sex.

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AUTHOR CONTRIBUTION

Nurten Aydogdu, Nesrin Emre and Ali Aydogdu contributed to the conception, coordination and design of the study. Nesrin Emre 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 diplozoid parasite species. Nurten Aydogdu, Ali Aydogdu and Özgür Emiroglu 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. Nurten Aydogdu and Ali Aydogdu participated in its design and coordination and helped to draft the manuscript. Nurten Aydogdu and Ali Aydogdu critically oversaw the substantial revisions of the manuscript.

CONFLICT OF INTEREST STATEMENT

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

ETHICAL APPROVAL

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 the live animal. All sampling and laboratory work on fish have complied with the Republic of Türkiye Ministry of Agriculture and Forestry Animal Welfare Laws.

DATA AVAILABILITY

Data supporting the results are available in the manuscript.

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