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First report of leech parasitism in freshwater turtles for Turkish wetlands

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ABSTRACT

Research on the diversities of parasites and hosts, as well as their relationships, can provide significant contributions to understanding, utilizing, conserving, and properly managing aquatic ecosystems. The freshwater mud turtle *Emys orbicularis* and Balkan turtle *Mauremys rivulata*, as well as the leech *Placobdella costata*, are distributed in Turkish wetlands; however, the relations between these organisms are not well-known. In this study, the parasitism of Glossiphonid leech *P. costata* on freshwater turtles, *E. orbicularis*, and *M. rivulata* was assessed for the first time through field observations and laboratory examinations in Turkish wetlands. The infection prevalence in turtles was found to be 76%, with an intensity of 5.00 and an abundance of 3.82, all of which were higher in females. Although the infection prevalence was similar for both *E. orbicularis* and *M. rivulata*, infection intensity and abundance were higher in *M. rivulata*. Leeches were mostly sampled from the plastron and at the least from the head. The condition of the parasitic leech was found to be high, and *P. costata* preferred to feed on and parasitize predominantly female individuals of both turtle species.

Keywords: *Placobdella costata*, *Emys orbicularis*, *Mauremys rivulata*, infection prevalence, infection intensity, abundance.

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Türkiye'deki sulak alanlar için tatlı su kaplumbağalarında sülük parazitliğine ilişkin ilk rapor

Öz: : Parazitlerin ve konakçıların çeşitliliğinin yanı sıra aralarındaki ekolojik ilişkilerin araştırılması, sucul ekosistemlerin anlaşılması, kullanılması, korunması ve uygun şekilde yönetilmesine önemli katkılar sağlayabilmektedir. Benekli kaplumbağa *Emys orbicularis* ve Balkan kaplumbağası *Mauremys rivulata* ile *Placobdella costata* sülük türü Türkiye sulak alanlarında yayılış göstermektedir; ancak bu organizmalar arasındaki ilişkiler tam olarak bilinmemektedir. Bu çalışmada, Glossiphonid sülüklerden *P. costata* türünün tatlı su kaplumbağaları, *E. orbicularis* ve *M. rivulata* üzerindeki parazitliği, saha gözlemleri ve laboratuvar incelemeleri yoluyla Türkiye sulak alanlarında ilk kez incelenmiştir. Kaplumbağalardaki enfeksiyon prevalansı %76, yoğunluğu 5,00 ve bolluğu 3,82 olarak bulunmuştur ve bunların hepsi dişilerde daha yüksek bulunmuştur. Enfeksiyon prevalansı hem *E. orbicularis* hem de *M. rivulata* için benzer olmasına rağmen, enfeksiyon yoğunluğu ve bolluğunun *M. rivulata* türünde daha yüksek olduğu belirlenmiştir. Sülükler en çok plastrondan, buna karşın en az ise kafadan örneklenmiştir. Sülüklerin kondisyonlarının yüksek olduğu ve *P. costata* türünün her iki kaplumbağa türünün ağırlıklı olarak dişi bireylerini parazitlemeyi tercih ettiği dikkat çekmiştir.

Anahtar kelimeler: *Placobdella costata*, *Emys orbicularis*, *Mauremys rivulata*, enfeksiyon prevalansı, enfeksiyon yoğunluğu, bolluk

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Introduction

Parasites play a crucial role in shaping ecosystems by influencing host behavior and fitness, trophic interactions, food webs, competition, biodiversity, and keystone species. This indicates that parasites are vital components that shape the structure of communities and ecosystems (Preston and Johnson 2010; Friesen et al. 2020). The study of parasite diversity is particularly significant due to the potential importance of sympatric diversity in certain parasite taxa and the opportunity to independently test evolutionary hypotheses in the numerous distinct lineages where parasitism has evolved (Poulin and Morand 2020). Parasites are well known for utilizing their hosts as a source of food and habitat (Siddall and Burreson 1996; O'Keeffe et al. 2021).

The diverse functions of leeches, including parasitic species, are of great importance in aquatic ecosystems. They are known for their therapeutic effects, special enzyme production, and use as raw materials for drug and cream production. Additionally, they serve as transmission vectors for numerous pathogens and can be used as wetlands quality indicators and for estimating of aquatic biodiversity (Siddall and Desser 1992; Kazancı et al. 2015; Sig et al. 2017; Williams et al. 2020; Kermanian et al. 2022; Lynggaard et al. 2022).

Freshwater turtle leech, *Placobdella costata*, is widely distributed in Euro-Asia, North Africa, and Turkey, in areas where its hosts are found. It is exclusively an ectoparasite of turtles (Sağlam 2001; Bielecki et al. 2007; Ayaz et al. 2008; Fediras et al. 2017), although it may also have other hosts, such as birds, amphibians, or reptiles. The reproductive period of *P. costata* coincides with its host's migration to breeding grounds, facilitating the dispersal of the parasite (Bielecki et al. 2012).

Turtles play a vital role in maintaining the health of aquatic ecosystems, making them significant organisms in wetland environments (Santori et al. 2020). In Turkey, the European pond turtle (*Emys orbicularis*) is found in freshwaters in Thrace, as well as in western and central Anatolia. Unfortunately, this species has experienced population decline due to the destruction and drying out of their natural habitats (Baran et al. 2005). *E. orbicularis* is also facing challenges in Europe, primarily due to anthropogenic habitat changes (Bielecki et al. 2007). The International Union for Conservation of Nature (IUCN) has listed this species as lower risk/near threatened (NT) globally (Çiçek et al. 2015). The Balkan's striped turtle (*Mauremys rivulata*) inhabits lakes, slow-moving waters, ditches, and wetlands. It is found in Thrace, as well as western and southern Anatolia. This species feeds on small invertebrates, aquatic insects, fish, and frogs (Baran et al. 2005; Ayaz and Budak 2008).

Leeches are a commonly observed parasitic organism affecting freshwater turtles (McCoy et. al. 2007; Readel et al. 2008). Several factors determine leech parasitism on turtles, including the species of both the leech and turtle, microclimate usage, sex, reproductive stage, body size, and environmental characteristics such as the month of capture, turtle abundance, vegetation, turbidity, wetland size, and availability of basking structures. The prevalence of leeches on turtles shows significant variation among turtle species, with the highest incidence observed in bottom-walking and adult turtles, throughout the year. Furthermore, leech intensity is highest in larger turtles and in turbid waters (Readel et al., 2008).

The aims of the study was to investigate occurrence of leech parasitism on freshwater turtles and to evaluate infestation prevalence and densities, abundance, species preferences, sex, attachment sites of body in wetlands around Lake Eğirdir, the second largest freshwater lake of Turkey.

Materials and Methods

Study Area

This research was conducted in the wetlands Aşağı Tırtar (38°14'31.8"N, 30°53'27.3"E), Boyalı (38°04'31.0"N, 30°51'12.4"E), Gelendost (38°02'56.0"N, 30°57'14.6"E), Kayaağzı (38°08'30.3"N, 30°45'12.8"E), and Akbük (38°09'11.9"N, 30°51'11.0"E), which are sublacustrine regions of Lake Eğirdir that are actively connected to the lake. These habitats are characterized as shallow and partially isolated from the lake and are covered by dense macrophytes. The fauna of these wetlands includes various aquatic, amphibian, and terrestrial animal species, such as freshwater turtles, waterfowls, frogs, snails, fish, wild boar, horse, donkey, dogs, wild cats, water vole, leeches, and others (Ceylan 2016).

Turtle and Leech Sampling

The sampling procedures were carried out on a monthly basis in 2013 and seasonally in 2014. The turtles were manually collected and subsequently transported to the laboratory for identification of species, determination of sex, measurement of body size and weight, and examination for leeches. The identification of turtle species was performed in accordance with Baran and Atatür (1998), while the determination of sex was carried out using eye color and plastron shape, as described by Zuffi and Gariboldi (1995), Ayaz et al. (2008), and Kaviani and Rahimibashar (2015).

Once in the laboratory, the shells and soft-tissue extremities of the turtles were carefully examined for leeches. Any leeches found on the turtles were extracted and fixed in 70% ethanol, without contraction, by gradually dripping the solution into a petri dish. Identification of the leeches was carried out under a stereo microscope, following the procedures described by Sawyer (1986) and Nesemann and Neubert (1999). The body length (L, cm) and weight (W, g) of each leech were determined, and the condition factor was calculated using the formula $K=W/L^3 \times 100$, as described by Ceylan et al. (2017). Following the completion of all procedures, the turtles were returned to the habitats from which they were collected.

Indicators of Parasitism

To determine the indicators of parasitism such as infestation prevalence, infestation density, and

abundance, the formulas provided by Laghzaoui et al. (2020) were utilized:

Infestation prevalence = (Number of turtles with leeches / Total number of turtles) x 100

Infestation density = Number of leeches / Number of turtles with leeches

Abundance = Number of leeches / Total number of turtles

Statistical Analyses

Descriptive statistics were computed, and normality of the data was assessed using the Shapiro-Wilk test (for n<30). The Mann-Whitney U test and Kruskal-Wallis H test were utilized to compare turtle species, sex, and the preferred body parts of the leech. Correlations were evaluated using the Spearman Correlation method. Values were reported as the mean ± standard deviation (Mean ± SD). The data were analyzed using IBM SPSS Statistics version 25.0 software package for Windows (IBM Corp., Armonk, NY, USA) with a significance level of alpha set at $\alpha = 0.05$.

Ethical Statement

The research was conducted with the endorsement of the Animal Experiments Local Ethics Committee of the Mediterranean Fisheries Research, Production, and Training Institute (Date: 29.02.2012, ID: 01-227).

Results

Turtles

During the study, a total of 17 turtles, belonging to two different species, were sampled primarily in May (Figure 1 and Table 1). Of the 17 turtles sampled, 13 were *Emys orbicularis* (Linnaeus, 1758) and four were *Mauremys rivulata* (Valenciennes, 1833). Fifteen turtles were in good condition, while two of them were deceased. Means of body weight, carapace length, carapace width and carapace height were 446±333 g, 13.3±3.7 cm, 9.4±2.5 cm, and 5.5±1.6 cm for *E. orbicularis*, and 574±569 g, 15.8±6.6 cm, 10.6±3.0 cm and 6.1±2.4 cm for *M. rivulata*, respectively. Among the *E. orbicularis* turtles, 76.9% were female and 23.1% were male; however, the difference was not statistically significant (Chi Square, P>0.05). In *M. rivulata*, an equal sex ratio was observed.

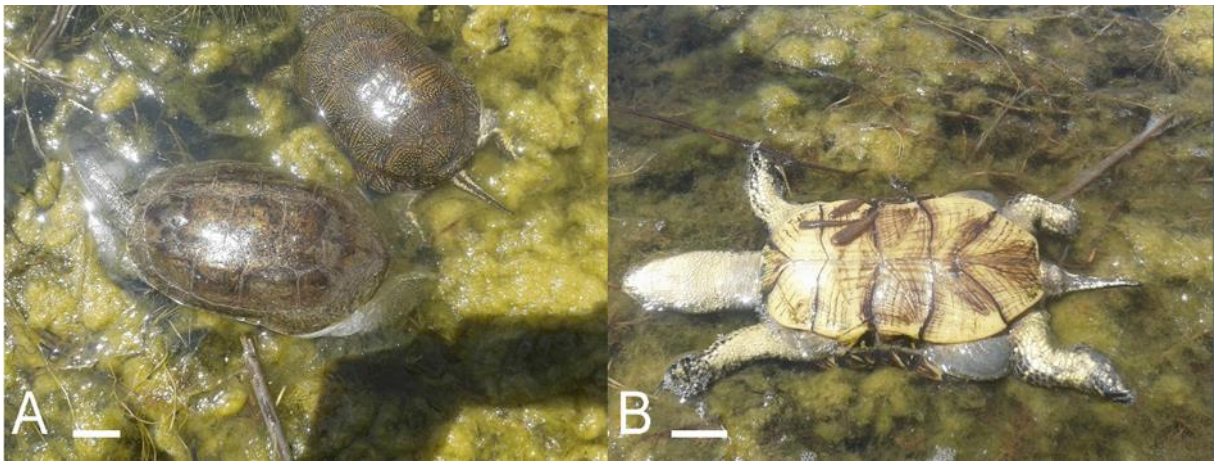

Figure 1. A: Turtles *Emys orbicularis* (top) and *Mauremys rivulata* (bottom) were found dead in the Boyalı wetland of Lake Eğirdir. B: Leeches of *Placobdella costata* in the plastron of *Emys orbicularis*. Scale = 1 cm.

Table 1. Monthly leech parasitism in turtle species*

	Months											
Turtle species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
E. orbicularis				3(7)	4(10)	1(6)	1(2)		1(2)			
M. rivulata							2(6)				1(32)	

* The figures situated outside the parentheses denote the count of turtles that have been parasitized by leeches, whereas the numbers within the parentheses indicate the overall quantity of leeches.

The morphological features of *E. orbicularis* include a carapace that is swollen and round, with a layer of skin in between that is not fully fused with the plastron. The carapace is typically black or brown, with yellowish spots and small stripes, while the plastron can vary from black to yellowish in color. Additionally, there are small yellow spots on the legs, tail, and neck of the species.

The morphological features of *M. rivulata* include a fused carapace and plastron on the sides. The carapace displays varying shades ranging from light to dark olive green, and different shades of

brown. Meanwhile, the plastron may appear either black or dark brown. The head, neck, and feet of *M. rivulata* exhibit black coloration with yellowish stripes present on the head and feet.

Leeches

All of the leeches found on the turtles were identified as *Placobdella costata* (Fr. Müller, 1846) (Figure 2). A total of 65 leeches were detected on the turtles, with 27 found on *E. orbicularis* and 38 on *M. rivulata*. Of the two deceased turtles that were sampled, one (*M. rivulata*) had no leeches, while the other (*E. orbicularis*) had three leeches in the plastron region. The average occurrence of *P. costata* was similar for both turtle species ($p>0.05$), indicating that *P. costata* showed no preference between the two turtle species.

Figure 2. Dorsal (left) and ventral (right) views of *Placobdella costata* sampled from turtles. Scale = 0.5 cm.

According to our findings, leeches were found to be primarily (48%) colonized in the plastron of the turtles, and significantly more frequent compared to other body parts (average of 9 leeches, $p<0.05$). The remaining body parts, namely the hind leg (20%), carapace (17%), foreleg (12%), and head (3%) exhibited no significant differences when compared to the plastron (Table 2). In addition, our results showed that leeches parasitized predominantly female individuals, as 56 out of 65 leeches (86%) were sampled from female turtles.

Table 2. The number of *P. costata* in different body parts of the turtles

		Body parts					
Turtle species	Sex	Plastron	Carapace	Head	Hind leg	Foreleg	Total
E. orbicularis	Female	8	2	2	6	4	22
	Male	2	0	0	3	0	5
Total of species		10	2	2	9	4	27
M. rivulata	Female	17	9	0	4	4	34
	Male	4	0	0	0	0	4
Total of species		21	9	0	4	4	38
Total of turtles		31	11	2	13	8	65

The body weight, length, and condition factor of the leeches were determined to be 0.11±0.07 g, 1.71±0.45 cm, and 2.171±1.102, respectively. A significant positive correlation was found between the body weight and length of the leeches ($r = 0.701$, $p<0.01$), and the length-weight relationship was estimated as $W=0.356*L^{1.962}$ (n=25). Additionally, the growth pattern of the leeches was determined to be negative allometric.

The morphological features of *P. costata* are as follows: the dorsal surface is convex, while the ventral surface is partly concave. The head is slightly broader, but not distinct from the body. The oral cavity is located at the anterior margin of the anterior sucker, and the posterior sucker is small. There are two pairs of eyes, which are often fused. The median segments consist of three rings. The genital pores are separated from each other by two annuli. Live individuals typically have a dark greenish-brown color with a row of white spots along the body margin. On the dorsal side, there are five longitudinal bands with prominent papillae, one of which is located in the center and has four dark spots. The other two pairs of stripes are paramedian and have prominent papillae, as shown in Figure 2.

Indicators of Parasitism

The infection prevalence among all turtles was determined to be 76.4%, with 77% in *E. orbicularis* and 75% in *M. rivulata*. The infection intensity was found to be 2.70 in *E. orbicularis* and 12.67 in *M. rivulata*, while the abundance was 2.08 in *E. orbicularis* and 9.50 in *M. rivulata*. In both species, the infection prevalence, infection intensity, and abundance values were higher among female turtles than males, as shown in Table 3.

Table 3. Infection prevalence, infection intensity and abundance of *P. costata* in turtles

Turtle species	Sex	Infection prevalence (%)	Infection intensity	Abundance
E. orbicularis	Female	80	2.75	2.20
	Male	67	2.50	1.67
General of species		77	2.70	2.08
M. rivulata	Female	100	17.00	17.00
	Male	50	4.00	2.00
General of species		75	12.67	9.50
General for turtles		76	5.00	3.82

Discussion

The distribution of two freshwater turtle species, *E. orbicularis* and *M. rivulata*, and the leech *P. costata*, is well documented in Turkish wetlands (Kazancı et al. 1992; Sağlam 2001; Özbek and Sarı 2007; Ayaz and Budak 2008, Bayrakcı et al. 2017). However, the parasitism of the Glossiphonid leech *P. costata* on these two freshwater turtle species, *E. orbicularis* and *M. rivulata*, has not been previously assessed in Turkish wetlands. Therefore, this study presents the first field observation and laboratory examination of this parasitic relationship.

The morphology of *P. costata* was consistent with previous reports in various habitats (Sawyer 1986; Neubert and Nesemann 1999; Elliott and Dobson 2015). Predominantly, the leeches found on the turtles were attached to the plastron, followed by the legs, carapace, and the head region (head and neck). Different attachment sites have been documented for the same leech by Bielecki et al. (2007), Bashirichelkasari and Yadollahvandmiandoab (2017), and Fediras et al. (2017). No significant correlation was found between parasite density and turtle size. The main peak of parasitism was observed in May and July, likely related to the rise in water temperature, feeding, and reproductive activity (Bielecki et al. 2012). Infection prevalence was found to be higher than in some other aquatic habitats (Bielecki et al. 2012; Fediras et al. 2017).

Aquatic vegetation cover, which serves as both shelter and a food source, is a crucial factor in determining the spatial distribution of turtles (Lebboroni and Chelazzi 1991). The wetlands surrounding Lake Eğirdir, with their abundant and healthy aquatic vegetation cover, as well as an ample supply of prey such as fish and frogs, provide an advantageous habitat for turtles and their parasitic leeches. However, turtle populations are facing significant threats from habitat destruction, over-collection for food and pets, and climate change (Bielecki et al. 2012; Lovich et al. 2018).

Three live *P. costata* were found on the deceased turtle, while the cause of death of the turtles is unclear. Previous studies have reported scavenging feeding characteristics of predatory leech species

(Davies et al. 1997; Pfeiffer et al. 2005; Ceylan et al. 2017). However, this finding needs to be analyzed in more details.

Further investigation, including hematological, histological, and biochemical analyses, as well as examination of host-parasite relationships, is necessary to fully understand the parasitism of *P. costata* in freshwater turtles.

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