Turkiye Parazitol Derg 2023;47(1):22-7
DOI: 10.4274/tpd.galenos.2022.38257

Prevalence of *Haemogregarina stepanowi* and Assessment of Some Risk Factors in *Mauremys rivulata* (Valenciennes, 1833) Freshwater Turtles (Testudines: Geoemydidae)

Mauremys rivulata (Valenciennes, 1833) Türü Tatlı Su Kaplumbağalarında (Testudines: Geoemydidae) Haemogregarina stepanowi'nin Prevalansı ve Bazı Risk Faktörlerinin Değerlendirilmesi

Onur Ceylan[1], Çiğdem Gül[2], Nurşen Çördük[2], Nurcihan Hacıoğlu Doğru[2], Murat Tosunoğlu[2]

[1]Selçuk University, Faculty of Veterinary Medicine, Department of Veterinary Parasitology, Konya, Türkiye
[2]Çanakkale Onsekiz Mart University, Faculty of Science, Department of Biology, Çanakkale, Türkiye

Cite this article as: Ceylan O, Gül Ç, Çördük N, Hacıoğlu Doğru N, Tosunoğlu M. Prevalence of *Haemogregarina stepanowi* and Assessment of Some Risk Factors in *Mauremys rivulata* (Valenciennes, 1833) Freshwater Turtles (Testudines: Geoemydidae). Turkiye Parazitol Derg 2023;47(1):22-7.

ABSTRACT

Objective: The Balkan terrapin, *Mauremys rivulata,* is a freshwater turtle. This reptile is exposed to many environmental pollutants and some infectious agents, including *Haemogregarina stepanowi* parasite. This study was conducted to determine the microscopic prevalence of haemogregarine infection in *M. rivulata* caught in three different localities (Bozcaada, Gökçeada, and Dardanos) in Çanakkale province of Turkey, and assessment of some risk factors.

Methods: Twenty-four blood samples were collected, thin blood smears were prepared, and the presence of haemogregarine parasites microscopically was screened. Water samples were also taken from the habitats, and these samples were analyzed physiochemically and microbiologically.

Results: Morphological identification was made by detecting the sausage-shaped intra-cytoplasmic developmental stages of *H. stepanowi*, and thirteen of twenty-four turtles (54.2%) were found to be infected. The prevalence of *H. stepanowi* was detected as the highest (90.0%) in the Gökçeada district, where the water pollution is higher than in the other localities. A statistically significant relationship was observed between the distribution of the infection and the gender of the turtles, the temperature of the water, the number of faecal coliforms in water and the amount of dissolved oxygen in the water. A statistically significant difference was found between the localities in terms of the prevalence of *H. stepanowi* infection, and the infection was primarily detected in the Gökçeada district.

Conclusion: This study has significance in providing information regarding haemoparasitic diseases of freshwater turtle, *M. rivulata,* in Turkey.

Keywords: Blood parasite, Çanakkale, freshwater turtle, morphology, Türkiye

ÖZ

Amaç: Balkan çizgili kaplumbağası olarak bilinen *Mauremys rivulata*, bir tatlı su kaplumbağasıdır. Bu sürüngen, birçok çevresel kirleticiye ve *Haemogregarina stepanowi* paraziti de dahil olmak üzere bazı enfeksiyöz etkenlere maruz kalmaktadır. Bu çalışma, Çanakkale ilinde üç farklı lokasyonda (Bozcaada, Gökçeada ve Dardanos) yakalanan *M. rivulata* türü tatlı su kaplumbağalarında hemogregarin enfeksiyonunun mikroskobik prevalansını belirlemek ve bazı risk faktörlerini değerlendirmek amacıyla yapılmıştır.

Yöntemler: Yirmi dört adet kan örneği alınmış, sürme kan preparatları hazırlanmış ve mikroskobik olarak hemogregarin parazitlerin varlığı taranmıştır. Ayrıca habitatlardan su numuneleri de alınmış ve bu numunelerin fizikokimyasal ve mikrobiyolojik analizleri yapılmıştır.

Received/Geliş Tarihi: 31.05.2022 **Accepted/Kabul Tarihi:** 08.09.2022

Address for Correspondence/Yazar Adresi: Onur Ceylan, Selçuk University, Faculty of Veterinary Medicine, Department of Veterinary Parasitology, Konya, Türkiye
Phone/Tel: +90 332 223 35 98 **E-mail/E-Posta:** onurceylan@selcuk.edu.tr **ORCID ID:** orcid.org/0000-0002-3514-5221

Türkiye PARAZİTOLOJİ Dergisi

Bulgular: *H. stepanowi*'nin sosis şeklindeki intrasitoplazmik gelişim evreleri tespit edilerek morfolojik tespit yapılmış ve 24 kaplumbağadan 13'ünün (%54,2) enfekte olduğu belirlenmiştir. *H. stepanowi* prevalansı su kirliliğinin diğer yerlere göre daha yüksek olduğu Gökçeada ilçesinde en yüksek düzeyde (%90,0) tespit edilmiştir. Enfeksiyonun dağılımı ile kaplumbağaların cinsiyeti, suyun sıcaklığı, sudaki fekal koliform sayısı ve sudaki çözünmüş oksijen miktarı arasında istatistiksel olarak anlamlı bir ilişki tespit edilmiştir. *H. stepanowi* enfeksiyonu prevalansı açısından lokaliteler arasında istatistiksel olarak anlamlı fark saptanmış olup, enfeksiyon başlıca Gökçeada ilçesinde tespit edilmiştir.
Sonuç: Bu çalışma, tatlı su kaplumbağası *M. rivulata*'nın Türkiye'deki hemoparaziter hastalıkları hakkında bilgi vermesi açısından önem taşımaktadır.
Anahtar Kelimeler: Hemoparazit, Çanakkale, tatlı su kaplumbağası, morfoloji, Türkiye

INTRODUCTION

Many factors can be listed as the reasons for the decrease in the number of reptile animals. Habitat loss and degradation, invasive species entering the habitats that alter the habitat' structure, fires, environmental pollutants such as agricultural pesticides, industrial wastes, radioactive and anthropogenic materials, diseases, the use of such animals for food and medicine in some Asian countries, and global climate change are the most important of these factors (1-3).

Parasitic diseases have a significant role among infectious diseases contributing to the decrease in the number of reptiles. Haemogregarine parasitic protozoa (Apicomplexa: Coccidia: Eucoccidiorida: Adeleorina) are intracellular haemoparasites causing infections, especially in reptiles such as lizards, turtles, snakes, or frogs. Turtles living in or near freshwaters are the most adversely affected reptiles by these parasitic agents (4-6). Haemogregarine parasites cause various clinical symptoms in freshwater turtles during their development. In the cases of haemogregarine infections, general weakness, anorexia, necrotic ulcerations on the skin and shell, decrease in motility, and skin hemorrhages may be encountered as indicators of poor health conditions (7).

Haemogregarine parasites have complex heteroxenous life cycles, including an indirect life cycle between invertebrate and vertebrate hosts. The parasites undergo asexual merogony and gametocyte formation stages in invertebrate hosts, mainly leeches, while they pass through sporogony and sexual gamogony stages in vertebrate hosts (8-10). Transmission occurs during leeches sucking blood from vertebrate hosts or when leeches are ingested by vertebrate hosts (8). Vertical transmission may also be a transmission route (11). These parasites infect erythrocytes and rarely leukocytes, and sausage-shaped intra-cytoplasmic gametocytes can easily be detected within these cells (5,9).

Freshwater turtles living in small rivers, ponds, dams, and other muddy environments are pollution indicators since the turtles act as water purifiers by reducing algal bloom (12). *Mauremys rivulata* (Valenciennes, 1833), one of the freshwater turtles, is listed in Appendix II of the Convention of European Wildlife and Natural Habitats of 1979 (13). This species is distributed in Thrace, Western and Southern Anatolia in Turkey. *M. rivulata* lives in natural and artificial habitats such as streams, seasonal ponds, lagoons, drainage and irrigation canals, dams, and reservoirs (14). Turkey, which acts as a wide land bridge between the continents of Africa, Asia, and Europe, is located at the intersection of the biota of these continents due to its geographical location and has wide diverse herpetofauna (15). Although many studies concerning the freshwater turtles, which are a significant part of the herpetofauna of Türkiye, have been conducted (16), the number of studies on haemoparasitic diseases of the amphibian and reptilian animals constituting the herpetofauna of Türkiye is insufficient.

This study was planned to determine the prevalence of a haemogregarine haemoparasitic agent *Haemogregarina stepanowi* in freshwater turtles of the species *M. rivulata* caught in the centre (Dardanos), Bozcaada and Gökçeada districts in Çanakkale province, and to evaluate the effects of various risk factors on the prevalence.

METHODS

Study Areas

The study area consists of 3 localities; Bozcaada (Azmak stream located in Çayır area 35S417612, 4410562), Gökçeada (Kaleköy area 35T0405784, 4453706), Çanakkale city center (Dardanos area 35T445711, 4438009) which are located in Marmara Region of Turkey. A map of sampling localities is indicated in Figure 1.

Physicochemical and Microbiological Analysis of Water Samples

Water samples were taken from each locality once and evaluated regarding physicochemical characteristics and microbial pollution (17). For this goal, water temperature, pH, electrical conductivity, and dissolved oxygen were measured *in situ* with the Hatch Lange trademark ecological kit. Total coliform, faecal coliform, and enterococcus were determined by the standard most probable number method (18).

Sample Collection, Clinical Examination, Species Identification of Captured Turtles and Carapace Measurements

During the field studies on the wetlands at each station, five turtles (5♀) from Bozcaada, nine turtles (3♂, 6♀) from Dardanos and ten turtles (5♂, 5♀) from Gökçeada were caught with the help of a net in April-July 2019. Captured turtles were macroscopically examined, but no health issues were observed. According to the morphological characteristics, all freshwater turtles caught in the present study were identified as the species *M. rivulata* belonging

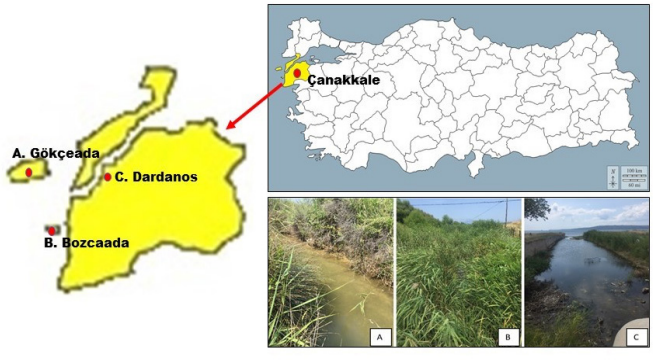

Figure 1. Map of Çanakkale province of Türkiye depicting the sampling localities

to the family Geoemydidae. The genders of the turtles were also determined to compare the prevalence of infection among genders. Additionally, the carapace length of the turtles was measured with a digital calliper, and the average carapace length for each locality was calculated (Bozcaada: 16.7 cm, Dardanos: 16.4 cm, Gökçeada: 19.7 cm).

Preparation of Blood Smears and Microscopic Examination

Two milliliters of blood sample were taken from the dorsal caudal vein of each turtle with the aid of a 5 mL syringe with a diameter of 21 needles. The stage of blood collection from turtles is shown in Figure 2. Thin blood smears were prepared from each blood sample and stained by Wright's staining method. Then, all smears were examined under a light microscope (Olympus CX 31) using 100x magnification, and the identification was made by detecting different developmental stages of the intraerythrocytic parasites (Figure 3) according to the relevant literature (10,19).

Ethical Statement

All experimental procedures were performed following the ethical guidelines of the Local Ethics Committee of Çanakkale Onsekiz Mart University (approval reference number: HADYEK, 2018/09-06). After morphological measurements and taking blood samples, turtles were released to the areas where they were collected.

Statistical Analysis

SPSS version 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical program was used to analyze all data. Fisher's Exact test was applied because the number of samples was less than 25. P-values were calculated to determine statistical significance among different gender of turtles and samples from different localities. Moreover, the prevalence value of *H. stepanowi* infection was statistically evaluated according to some water parameters where the turtles live, such as the number of faecal coliforms, temperature, and dissolved oxygen.

Figure 2. The stage of collecting blood sample from a freshwater turtle

RESULTS

The obtained data regarding physicochemical characteristics such as temperature, pH, electrical conductivity, and dissolved oxygen of water samples and some microbiological parameters measured during the study are indicated in Table 1.

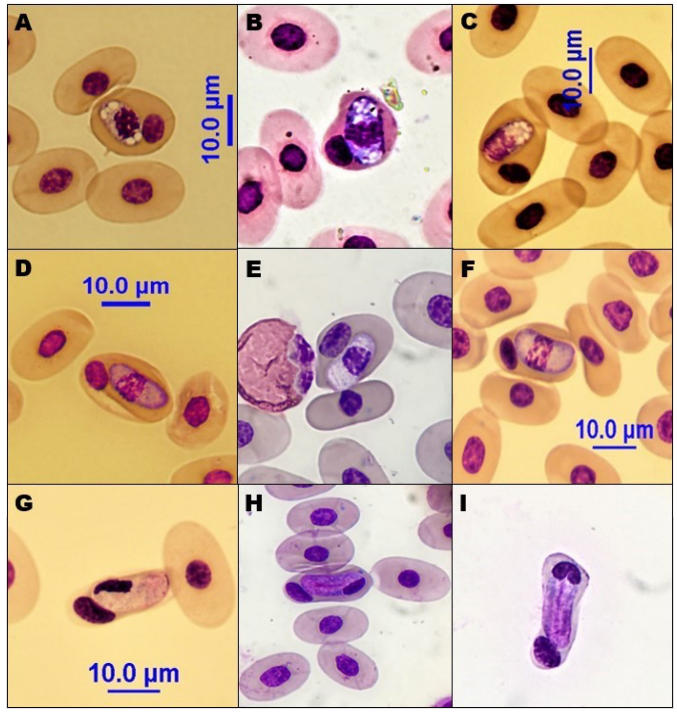

Figure 3. Photomicrographs of intraerythrocytic stages of *Haemogregarina stepanowi* Danilewsky, 1885 from *Mauremys rivulata* (Wright stain method) (X100 magnification), Trophozoites including many vacuoles (A-C), premeronts of *Haemogregarina stepanowi* with centric erythrocyte nucleus (without vacuoles) (D-F), different morphotypes of mature gamonts (G-I)

Table 1. Physicochemical and microbiological characteristics of water samples from three localities

Parameter	Bozcaada	Gökçeada	Dardanos
pH	8.11 (I-II)	7.54 (I-II)	8.02 (I-II)
Temperature (°C)	25.5 (II)	25.7 (II)	29.8 (III)
Electrical conductivity (µS/cm)	3.37 (I)	987 (I)	3.01 (I)
Dissolved O_2 (mg/L)	7.43 (I)	0.13 (IV)	7.82 (I)
Total coliform (MPN/100 mL)	$4x10^2$ (I-II)	$2x10^3$ (I-II)	0 (I)
Faecal coliform K (MPN/100 mL)	$9x10^1$ (I-II)	$9x10^2$ (II-III)	0 (I)
Faecal enterococ S (MPS/100 mL)	0	$9x10^2$	$3x10^2$

According to microbiological measurements performed in the study, it has been determined that three localities have I. quality water (high-quality water) in terms of total coliform counts. However, it has been determined that Bozcaada and Dardanos are included in the first quality water classes and Gökçeada in the second (slightly contaminated water) or third (contaminated water) quality water classes in terms of faecal coliform count. In addition, the Gökçeada district was found the most polluted locality, and the anthropogenic burden was felt highest in Gökçeada.

Altogether, 13 of 24 (54.2%) freshwater turtles of *M. rivulata* species were found to be infected with *H. stepanowi* in the study. The highest prevalence was detected in the Gökçeada district (90%). Infection rates according to sample collection areas and genders of the turtles are shown in Table 2.

In the study, the effects of various factors on the distribution of *H. stepanowi* infection were statistically analyzed. It was determined that gender, locality, some parameters concerning water such as temperature, dissolved oxygen, and the number of faecal coliforms associated with haemogregarine infections in *M. rivulata* specimens. Infection was detected more prevalent in male turtles than in females in the study, and this situation was found statistically significant ($p<0.05$). A statistically significant difference was also found between the localities in terms of the prevalence of infection, and the infection was primarily detected in the Gökçeada district ($p<0.05$). It is thought that this situation is caused by the microbiological quality characteristics of the water in the Gökçeada district because the total coliform, faecal coliform, and faecal enterococ counts measured in this district were found to be higher than in the other two localities. When the faecal coliform count causing significant differences in the water quality rankings of the localities was subjected to statistical analysis, a statistically significant relationship was found between the number of faecal coliforms and the prevalence of infection ($p<0.05$). In addition, a significant relationship was determined in terms of the distribution of infection according to the water temperature. The infection rate was higher in Bozcaada and Gökçeada, where the water temperature was lower ($p<0.05$). Finally, *H. stepanowi* infection was detected less prevalent in Bozcaada and Dardanos, where the dissolved oxygen amount in the water is higher, compared to Gökçeada, where the dissolved oxygen amount is very low. A statistically significant relationship was found between the prevalence of infection and dissolved oxygen in the water. Statistical analysis findings are shown in Table 3.

Table 2. Distribution of haemogregarin infection according to localities and genders

Localities	Number of captured turtles	Genders of turtles	Infection rate (%)
Bozcaada	5	5♀	2/5 (2♀) (40%)
Gökçeada	10	5♂, 5♀	9/10 (5♂, 4♀) (90%)
Dardanos	9	3♂, 6♀	2/9 (2♂) (22.2%)
Total	24	8♂, 16♀	13/24 (7♂, 6♀) (54.2%)

Table 3. The results of statistical analysis comparing the distribution of *H. stepanowi* infection according to different parameters

		H. stepanowi		Total	p
		-	+		
Gender	Male	1	7	8	0.033
	Female	10	6	16	
Total		11	13	24	
Locality	Dardanos	7	2	9	0.006
	Bozcada	3	2	5	
	Gökçeada	1	9	10	
Total		11	13	24	
Faecal coliform	I. quality	10	4	14	0.005
	II. and III. quality	1	9	10	
Total		11	13	24	
Temperature	II. quality	4	11	15	0.033
	III. quality	7	2	9	
Total		11	13	24	
Dissolved O_2	Low	1	9	10	0.005
	High	10	4	14	
Total		11	13	24	

DISCUSSION

Haemogregarine protozoa are the most commonly distributed haemoparasitic agents of a wide range of turtles, including freshwater turtles worldwide. The family Haemogregarinidae currently consists of the genera *Cryilia*, *Haemogregarina*, *Hepatozoon*, *Haemolivia,* and *Karyolysis*. *Haemogregarina stepanowi*, which belongs to the genus *Haemogregarina*, is one of the most important species causing infections in freshwater turtles. This species has been reported from several freshwater turtle species such as *Emys orbicularis*, *E. trinacris*, *M. caspica*, *M. rivulata* and *M. leprosa* (4,19). In the present study, *H. stepanowi* was detected with a prevalence value of 54.2 percent of *M. rivulata* specimens collected from 3 different localities in Çanakkale province located in the Marmara Region, Türkiye.

Many studies report *Haemogregarina* infections in freshwater turtles, and studies on freshwater turtles and other reptiles have increased in recent years (6,20,21). However, as a result of the literature review, it was determined that studies on haemogregarine infections of freshwater turtles in Türkiye are scarce. Dvořáková et al. (19) investigated the prevalence of *H. stepanowi* in western Palaearctic freshwater turtles of the *Emys* and *Mauremys* genera in a study conducted in Türkiye. The prevalence of *H. stepanowi* in freshwater turtle species of *M. caspica* and *E. orbicularis* collected from Diyarbakır province was determined as 33.3% and 100%, respectively. They also determined the prevalence of infection as 54.5% in *M. rivulata* species collected from three different localities (Balıkesir, Selçuk, and Kemer). In addition, they pointed out that *H. stepanowi* has a wide distribution from North Africa to Europe, from Türkiye and the Middle East to Iran (19). *Mauremys rivulata* species, which constitute the materials of this study, were collected from Çanakkale, which is geographically relatively close to the mentioned places, and the infection rate in *M. rivulata* freshwater turtles was found to be very close (54.2%) to the prevalence determined in the previous study (54.5%). This situation may be attributed to the similar habitats or climatic conditions in which freshwater turtles live.

Haemogregarine infections of freshwater turtles may differ from region to region depending on environmental pollution (22). The effects of different pollutants in two different areas on leech infestations and haemogregarine infections were investigated in *Phrynop geoffroanus,* which is commonly known as Geoffroy's side-necked turtle. It was noted that turtles living in urban areas exhibit a higher parasitism rate due to human impact on land use and domestic and industrial waste production (23). The effects of some factors on the prevalence of infections were also investigated by physicochemical and microbiological analyzes of the waters where the turtles are found in this study. The prevalence of *H. stepanowi* was found to be the highest (90%) in Gökçeada, where the highest microbiological water pollution was detected in the present study. In addition, a statistically significant association was found between the prevalence of *H. stepanowi* infection and the temperature of the water, and the amount of dissolved oxygen in the water. The prevalence was found to be higher in Bozcaada and Gökçeada, which have II. quality water according to water temperature, compared to Dardanos (III. quality). An inverse association was determined between the amount of dissolved oxygen in the water and the prevalence of infection. Accordingly, the highest infection rate was found in Gökçeada, with the lowest dissolved oxygen. Although it is a known fact that the mentioned parameters regarding water are seriously affected by various pollutants such as bacterial microorganisms, all these findings support the view that haemogregarine infections increase in freshwater turtles due to water pollution.

Another factor thought to be effective in the prevalence of the infection among individuals is the size of the turtles. Larger turtles have a wider surface area; therefore, leech infestations are more common in these turtles than in small ones. This situation also increases haemogregarine infection for larger turtles (24). In the present study, the higher prevalence of infection determined in the Gökçeada district, where the average length of carapace is the highest, supports this view. Although some studies show that the gender factor also affects the prevalence of the haemogregarine parasite in male and female turtles, different results have been obtained regarding this situation (22). Özvegy et al. (7) could not detect a statistically significant difference in the prevalence of *Haemogregarine* spp. between male and female turtles. The prevalence value in male turtles was found to be higher, with a statistically significant difference in this study.

Haemogregarine parasites can cause a decrease in haemoglobin concentration in erythrocytes resulting in insufficient oxygen transportation to tissues (25). Depending on the severity of infection, the general health status is adversely affected, and symptoms such as weakness, reluctance to move, and anorexia may occur in turtle populations exposed to haemogregarine parasites. Skin haemorrhages and necrotic ulcerations on the shell and skin are other symptoms that can be seen in severe infections (7,26). No macroscopic abnormalities or signs of haemogregarine infection were detected in the turtles examined in this study. This situation is thought to be due to the low parasitemia detected in microscopic examination.

CONCLUSION

This study will contribute to expanding the knowledge about the prevalence and epidemiology of *H. stepanowi* infections in freshwater turtles whose haemoparasitic infections have been rarely studied in Türkiye. The overall microscopic prevalence of *H. stepanowi* was 54.2% in apparently healthy *M. rivulata* freshwater turtles. It was statistically determined that some water-related parameters and gender played an important role in the distribution of infections in the present study. The prevalence value was found to be the highest in the Gökçeada district, where the water pollution and carapace length of turtles were the highest. Increasing environmental pollution caused by urbanization negatively affects the lives of many reptilian animals comprising Turkey's herpetofauna. In order to prevent this situation, further epidemiological studies covering different geographical regions and detailed studies assessing more risk factors are required.

*** Acknowledgment**

This study was financially supported by the Scientific Research Project Coordination Unit of Çanakkale Onsekiz Mart University, Turkey (FBA-2018-2781).

*** Ethics**

Ethics Committee Approval: All experimental procedures were performed following the ethical guidelines of the Local Ethics

Committee of Çanakkale Onsekiz Mart University (approval reference number: HADYEK, 2018/09-06).

Informed Consent: Not required.

Peer-review: Internally and externally peer-reviewed.

*** Authorship Contributions**

Concept: O.C., Ç.G., Design: O.C., Ç.G., Data Collection or Processing: O.C., Ç.G., N.Ç., N.H.D., M.T., Analysis or Interpretation: O.C., Ç.G., N.Ç., N.H.D., M.T., Literature Search: O.C., Ç.G., N.Ç., N.H.D., M.T., Writing: O.C.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was financially supported by the Scientific Research Project Coordination Unit of Çanakkale Onsekiz Mart University, Turkey (FBA-2018-2781).

REFERENCES

1. Gibbons JW, Scott DE, Ryan TJ, Buhlmann KA, Tuberville TD, Metts BS, et al. The global decline of reptiles, Déjà vu Amphibians. Bioscience 2000; 50: 653-66.
2. Lee SY, Dunn RJK, Young RA, Connolly RM, Dale PER, Dehayr R, et al. Impact of urbanization on coastal wetland structure and function. Austral Ecology 2006; 31: 149-63.
3. Stokeld D, Hamer AJ, Van der Ree R, Pettigrove V, Gillespie G. Factors influencing occurrence of a freshwater turtle in an urban landscape: a resilient species? Wildlife Research 2014; 41: 163-71.
4. Al-Qurarishy S, Abdel-Ghaffar F, Dkhil MA, Abdel-Gaber R. Haemogregarines and criteria for identification. Animals (Basel) 2021; 11: 170.
5. Arıkan H, Çiçek K. Haematology of amphibians and reptiles: a review. North-West J Zool 2014; 10: 190-209.
6. Netherlands EC, Cook CA, Du Preez LH, Vanhove MPM, Brendonck R, Smit NJ. Monophyly of the species of *Hepatozoon* (Adeleorina: Hepatozoidae) parasitizing (African) anurans, with the description of three new species from hyperoliid frogs in South Africa. Parasitology 2017; 145: 1039-50.
7. Özvegy J, Marinković, D, Vučicević M, Gajić B, Stevanović J, Krnjaić D, et al. Cytological and molecular identification of *Haemogregarina stepanowi* in blood samples of the European pond turtle (*Emys orbicularis*) from quarantine at Belgrade zoo. Acta Vet-Beograd 2015; 65: 443-53.
8. O'Donoghue P. Haemoprotozoa: making biological sense of molecular phylogenies. Int J Parasitol Parasites Wildl 2017; 6: 241-56.
9. Paperna I. Developmental cycle of chelonian haemogregarines in leeches with extra-intestinal multisporozoite ocysts and a note on the blood stages in the chelonian hosts. Dis Aquat Org 1989; 7: 149-53.
10. Telford JSR. Hemoparasites of the Reptilia: Color Atlas and Text. New York, CRC Press, 2009.
11. Kauffman KL, Sparkman A, Bronikowski AM, Palacios MG. Vertical transmission of *Hepatozoon* in the Garter Snake Thamnophis elegans. J Wildl Dis 2017; 53: 121-5.
12. Khan MQ, Yaseen, Zahid H, Numan M, Da Silva Vaz Jr I, Ali A. Ecology and genetic identification of freshwater turtles in Pakistan. Acta Sci Vet 2021; 49: 1813.
13. Çördük N, Doğru N, Gül Ç, Tosunoğlu M. Assessment of nuclear abnormalities in erythrocytes of Balkan pond turtle *Mauremys rivulata* (Valenciennes, 1833) (Testudines: Geoemydidae) from the Biga stream, Canakkale, Turkey. Acta Zool Bulg 2019; 71: 219-26.
14. Baran İ, Avcı A, Kumlutaş Y, Olgun K, Ilgaz Ç. Türkiye Amfibi ve Sürüngenleri. Palme Yayınevi; 2021.
15. Cox N, Chanson J, Stuart S. The status and distribution of reptiles and amphibians of a Mediterranean Basin. UICN, Gland Switzerland and Cambridge; 2006.
16. Arslan D, Olivier A, Yaşar Ç, Ismail IB, Döndüren Ö, Ernoul L, et al. Distribution and current status of herpetofauna in the Gediz Delta (Western Anatolia, Turkey). Herpetol Notes 2018; 11: 1-15.
17. Hacıoğlu Doğru N, Gül Ç, Çördük N, Tosunoğlu M. Determination of the effects of environmental pollution on the Balkan terrapin, Mauremys rivulata (Valenciennes, 1833). Acta Vet Hung 2022; 70: 245-53.
18. Finstein MS. Pollution Microbiology a Laboratory Manual, Marcel Dekker, New York; 1972.
19. Dvořáková N, Kvičerová J, Papoušek I, Javanbakht H, Tiar G, Kami H, et al. Haemogregarines from western Palaearctic freshwater turtles (genera *Emys*, *Mauremys*) are conspecific with *Haemogregarina stepanowi* Danilewsky, 1885. Parasitology 2014; 14: 522-30.
20. Javanbakht H, Sharifi M. Prevalence and intensity of *Haemogregarina stepanowi* (Apicomplexa: Haemogregarinidae) in two species of freshwater turtles (*Mauremys caspica* and *Emys orbicularis*) in Iran. J Entomol Zool Stud 2014; 2: 155-8.
21. Rakhshandehroo E, Sharifiyazdi H, Ahmadi A. Morphological and molecular characterisation of *Haemogregarina* sp. (Apicomplexa: Adeleina: Haemogregarinidae) from the blood of the Caspian freshwater turtle *Mauremys caspica* (Gmelin) (Geomydidae) in Iran. Syst Parasitol 2016; 93: 517-24.
22. Hossen MS, Bandyopadhyay PK, Gürelli G. On the occurrence of a Haemogregarinae (Apicomplexa) parasite from freshwater turtles of South 24 Parganas, West Bengal, India. Turkiye Parazitol Derg 2013; 37: 118-22.
23. De Campos Brites VL, Rantin FT. The influence of agricultural and urban contamination on leech infestation of freshwater turtles, *Phrynops geoffroanus*, taken from two areas of the Uberabinha River. Environ Monit Assess 2004; 96: 273-81.
24. McCoy JC, Failey EL, Price SJ, Dorcas ME. An assessment of leech parasitism on semi-aquatic turtles in the western piedmont of North Carolina. Southeast Nat 2007; 6: 191-202.
25. Oppliger A, Célérier ML, Clobert J. Physiological and behaviour changes in common lizards parasitized by haemogregarines. Parasitology 1996; 113: 433-8.
26. Knotkova Z, Mazanek S, Hovorka M, Sloboda M, Knotek Z. Haematology and plasma chemistry of Bornean river turtles suffering from shell necrosis and haemogregarine parasites. Vet Med 2005; 9: 421-6.