Commagene Journal of Biology

Çördük et al., (2024) *Comm. J. Biol.* 8(1), 30-36.
DOI: 10.31594/commagene.1461253

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

Erythrocytic and Nuclear Abnormalities in *Natrix* sp. from the Biga Stream (Çanakkale, Türkiye)

Nurşen ÇÖRDÜK[1,*], Begüm BORAN[2], Bengi BAYCAN[2], İbrahim UYSAL[3]

[1]*Çanakkale Onsekiz Mart University, , Faculty of Sciences, Department of Biology, Çanakkale, TÜRKIYE*
[2]*Çanakkale Onsekiz Mart University, Department of Biology, School of Graduate Studies, Çanakkale, TÜRKIYE*
[3]*Çanakkale Onsekiz Mart University, Vocational School of Health Services, Çanakkale, TÜRKIYE*
ORCID ID: Nurşen ÇÖRDÜK: https://orcid.org/0000-0001-8499-4847; Begüm BORAN: https://orcid.org/0000-0002-3069-7780;
Bengi BAYCAN: https://orcid.org/0000-0002-2758-6166; İbrahim UYSAL: https://orcid.org/0000-0002-7507-3322

Received: 29.03.2024 **Accepted:** 12.06.2024 **Published online:** 30.06.2024 **Issue published:** 30.06.2024

Abstract: The Grass Snake (*Natrix natrix*) and the Dice Snake (*Natrix tessellata*) are potential bioindicator water snake species in certain aquatic habitats. The aim of this study is to evaluate the frequency of erythrocytic abnormalities, immature erythrocyte index, and nuclear abnormalities in *Natrix natrix* and *Natrix tessellata* from Biga Stream in Çanakkale, Türkiye. Erythrocytic abnormalities and nuclear abnormalities such as micronuclei, lobbed nuclei, notched nuclei, blebbed nuclei, and binucleate cells were observed in peripheral blood smears for each sample. According to our results, total erythrocytic abnormalities in *N. natrix* (3.01±0.55%) were higher than in *N. tessellata* (2.52±0.32%). Similarly, the frequency of total nuclear abnormalities in *N. natrix* (13.40±2.7%) was higher than in *N. tessellata* (12.68±5.81%) but there was no statistically significant difference in total erythrocytic and nuclear abnormalities. However, student's *t*-test revealed that there was a statistically significant difference between the two species only in terms of immature erythrocyte index (p: 0.008) which was higher in *N. natrix*. Due to the results, it was suggested that *N. natrix* may exhibit more physiological responses to envirenmental stress factors.

Keywords: Grass snake, dice snake, micronucleus test, ecotoxicology.

Biga Çayı (Çanakkale, Türkiye)'nda Dağılış Gösteren *Natrix* sp.'lerin Eritrositik ve Nüklear Anormallikleri

Öz: Yarı Sucul Yılan (*Natrix natrix*) ve Su Yılanı (*Natrix tessellata*), belirli sucul habitatlarda potansiyel biyoindikatör olan yılan türleridir. Bu çalışmanın amacı, Biga Çayı'nda (Çanakkale, Türkiye) bulunan *Natrix natrix* ve *Natrix tessellata* türlerinin eritrositik anormallik frekansı, olgunlaşmamış eritrosit indeksi ve nüklear anormallik frekansının belirlenmesidir. Her bir örnek için periferik kan yaymalarında, eritrositik anormallikler ve mikronükleus, loblu nükleus, çentikli nükleus, tomurcuklu nükleus, binükleat hücre gibi nüklear anormallikler gözlenmiştir. Sonuçlarımıza göre, *N. natrix*'teki toplam eritrositik anormallikler (%3.01±0.55) *N. tessellata*'dan (%2.52±0.32) daha yüksektir. Aynı şekilde *N. natrix*'teki toplam nüklear anormallik frekansının (%13.40±2.7) da *N. tessellata*'dan (%12.68±5.81) daha yüksek olduğu tespit edilmiştir fakat toplam eritrositik ve nüklear anormallikler bakımından istatistiksel olarak anlamlı bir fark bulunmamıştır. Ancak, student *t* testi ile iki tür arasında sadece olgunlaşmamış eritrosit indeksi (p: 0.008) açısından istatistiksel olarak anlamlı farklılık olduğu, *N. natrix*'de daha yüksek olduğu ortaya konmuştur. Bu sonuçlar doğrultusunda *N. natrix*'in çevresel stres faktörlerine karşı daha fazla fizyolojik tepkiler gösterebileceği düşünülmektedir.

Anahtar kelimeler: Yarı sucul yılan, su yılanı, mikronükleus testi, ekotoksikoloji.

1. Introduction

Assessing the health and function of ecosystems demands comprehensive data which may include the use of bioindicator species (O'Connor & Dewling, 1986; Paoletti & Sommaggio, 1996; Sharma & Rawat, 2009; Siddig et al., 2016). Large reptilian predators, such as snakes, are increasingly acknowledged as a valuable bioindicator to monitor ecosystem health (Manolis et al., 2002; Haskins et al., 2021; Lettoof et al., 2022). Water snakes consume both aquatic and terrestrial food sources and occupy a remarkable trophic level in their ecosystems so that these animals could accumulate environmental pollutants owing to their wide geographic range and population density (Valbona et al., 2015).

In Türkiye, there are two species within the *Natrix* genus: *N. natrix* and *N. tessellata*. *N. natrix* predominantly inhabits grassy and rocky areas closer to water. It can also be found in stagnant and flowing waters as well as in fields and gardens. *N. tessellata* lives in and around water bodies (Baran et al., 2012). In habitats where these two species coexist, *N. tessellata* predominantly inhabits aquatic environments and feeds almost exclusively on fish. In contrast, *N. natrix* prefers moist areas that are close to water and primarily preys on amphibians with larger individuals occasionally consuming small birds, rodents, or lizards (Hojati et al., 2013). *N. natrix* is observed in suitable habitats everywhere except for the Southeastern Anatolia Region while *N. tessellata* is observed in suitable habitats everywhere except for the eastern parts of the Eastern Black Sea and Inner Anatolia regions in Türkiye (Baran et al., 2012). According to the International Union for Conservation of Nature (IUCN) Red List for endangered species, both *N. natrix* and *N. tessellata* are assessed as "least concern" (LC). However, evaluations of the European populations of *N. tessellata* indicates decreasing population (IUCN, 2023). Previous studies that suggest abnormalities in the erythrocytes of *N. natrix*

Corresponding author: nursencorduk@comu.edu.tr

indicate the impact of cytotoxic and genotoxic contaminants (Valbona et al., 2015) and findings about metal bioaccumulation regarding environmental pollution caused by heavy metals in *N. tessellata* (Yermolenko et al., 2019) making these water snake species a bioindicator for assessing the health of freshwater ecosystems. There are some hematological (Tok et al., 2006; Tosunoğlu et al., 2011; Gül et al., 2018; 2019), plasma biochemical (Erdoğan & Tosunoğlu, 2017), and nuclear abnormality (Tok et al., 2014) studies conducted on *Natrix* species in Türkiye.

The examination of blood smears is an invasive and easy method that has proven effective in evaluating the health and physiological condition of animals in natural habitats (Arıkan & Çiçek, 2010; van der Horst et al., 2012). Recently, morphological alterations in erythrocytes can be used as significant biomarkers for assessing the impacts of environmental pollution (Strunjak-Perović et al., 2010). The alterations in erythrocyte morphology may serve as a supplementary method for predicting genotoxicity (Pollo et al., 2019). In addition to erythrocytic cell abnormalities, the presence of micronuclei formation and the occurrence of nuclear abnormalities were utilized as parameters to indicate the genotoxic effects (Carrasco et al., 1990; Nascimento et al., 2010; Marcussi et al., 2011; Valbona et al., 2015).

The increased variety and quantity of pollutants have necessitated the development of several bioassay test systems to evaluate the genotoxic effects caused by these agents in living organisms. Short-term genotoxicity tests are widely utilized to monitor the potential harm of various chemicals to humans, animals, and plants. Among these tests, the Micronucleus test (MN) is employed to assess the biological impacts of water pollutants on genotoxic damage in organisms dwelling in aquatic environments (Frenzilli et al., 2009; Bolognesi & Hayashi, 2011; Kousar & Javed, 2015). MN serves as a continuous and effective means to evaluate pollution levels in aquatic environments (Obiakor et al., 2012). However, recently, other nuclear abnormalities such as lobbed nuclei (LB), notched nuclei (NT), and blebbed nuclei (BL) have been identified and described to monitor the genotoxic effects of pollutants. Assessing the number of micronuclei alongside other nuclear abnormalities formed in the cells of various species exposed to different environmental pollutants can aid in evaluating the genotoxic effects of these pollutants (Strunjak-Perovic et al., 2010). In recent years, nuclear abnormalities have been combined with micronucleus analysis to assess the effects of pollutants in various species (Ergene et al., 2007; Guilherme et al., 2008; Napierska et al., 2009; Strunjak-Perovic et al., 2010).

The objective of this study was to assess the possible damages on erythrocytes inflicted by water pollution on *Natrix natrix* and *Natrix tessellata* from the Biga Stream (Çanakkale, Türkiye) as ophidian bioindicator oganisms by examining erythrocytic and nuclear abnormalities and immature erythrocyte index.

2. Material and Methods

2.1. Study area

Çanakkale is in the northwest of Türkiye with its lands extending over the Gelibolu Peninsula and the Biga Peninsula in Asia and Europe. One of the biggest streams in Biga Peninsula is Biga Stream. Due to its drainage areas intersecting with numerous settlements, Biga Stream is under the threat of pollution. Additionally, the stream is under pressure due to the expansion of nearby landfills and agricultural areas (Akbulut et al., 2006). Since both *Natrix* species (*N. natrix* and *N. tessellata*) coexist sympatrically in the Biga Stream, this study was conducted in a locality (40º22′23.00″ N; 27º18′37.51″ E) near the point where the stream flows into the sea (Fig. 1).

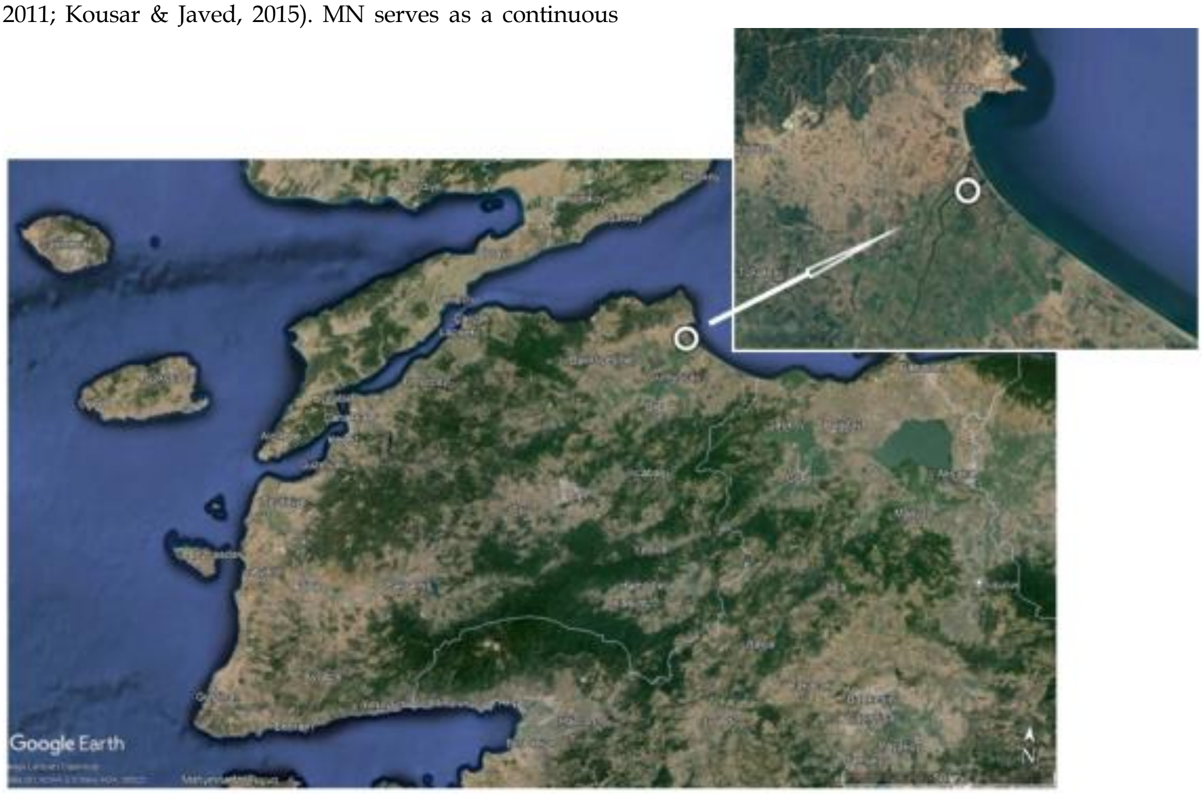

Figure 1. Study site.

2.2. Data collection

Natrix natrix (*n* =12) and *N. tessellata* (*n* =14) specimens were captured from the Biga Stream between May 2014 and 2017. Only healthy and adult animals were studied and they were collected by hand capture method generally from the water. The animal specimens were collected according to the guidelines of the Çanakkale Onsekiz Mart University's ethics committee (Decision numbers: 2012/05-01, 2017/04-16). Blood samples were collected using a 2 mL syringe with a 21-gauge needle from the postorbital sinus of specimens (Ballard & Cheek, 2003; Thrall et al., 2004). Blood smears were prepared from the samples and examined using an Olympus CX21 microscope. Following the blood collection, the specimens were brought back to their habitats.

2.3. Examination of blood smears

Erythrocyte morphology abnormalities, including pyknotic erythrocytes (PE), mitotic erythrocytes (ME), anucleated erythrocytes (AE), nuclear shift (NS), elliptical shape distortion (ESD), and vacuolisation (V) were assessed as indicators of hematological fluctuations (Pollo et al., 2019). The nucleus of a pyknotic erythrocyte is smaller compared to normal mature erythrocytes and anucleated and mitotic erythrocytes can be easily distinguished. Differentiation between mature (ME) and immature erythrocytes is based on two criteria such as cytoplasm color and nucleus size (Guilherme et al., 2008). Immature erythrocyte index (IE) was calculated by counting 1000 immature+mature erythrocytes on the blood smears according to Guilherme et al., 2008. Peripheral blood smears were prepared on clean slides for Micronucleus Test. Following fixation in ethanol for 20 minutes, the slides were air-dried at room temperature and subsequently fixed with methanol for 15 minutes and stained with Giemsa stain (10% v/v) (Josende et al., 2015). For each animal sample, three slides were prepared and a total of 1000 erythrocytes per slide were examined using an optical microscope at 1000x magnification. Micronuclei were identified based on specific criteria: (a) they should be smaller than one-third of the main nuclei, (b) they must not contact the main nuclei, and (c) they must not exhibit refractivity, displaying the same color and intensity as the main nuclei (Heddle & Countryman, 1976; Titenko-Holland et al., 1997; Fenech, 2000). Additionally, other nuclear abnormalities such as lobbed nuclei, notched nuclei, blebbed nuclei, and binucleated cells were observed and scored on the slides (Carrasco et al., 1990; Strunjak-Perovic et al., 2010; Josende et al., 2015).

2.4. Statistical analyses

Following the Shapiro-Wilk Test for normality, the student's *t* test for parametric data or Mann Whitney U test for non-parametric data was utilized to determine whether there was a difference in all values between species using SPSS 20.0 for PC (SPSS, Chicago, IL, USA). In all cases, $p \leq 0.05$ value was considered statistically significant. Following the analyses, no significant difference was observed between genders; hence, female, and male specimens were evaluated together.

3. Results and Discussion

The erythrocytic abnormalities (such as pyknotic, mitotic, anucleated erythrocytes, nuclear shift, elliptical shape disorders, and vacualisation, see Fig. 2) and immature erythrocyte index (IE) of two *Natrix* species (*N. natrix* and *N. tessellata*) living in the Biga Stream were given in Table 1. Student-*t* test was revealed that there was statistically significant difference between the two species only in the immature erythrocyte index (t: 2.881, df: 24, p: 0.008).

Table 1. Erythrocytic abnormalities (%) and immature erythrocyte index (IE) (‰) in *N. natrix* and *N. tessellata*.

	Natrix natrix			*Natrix tessellata*		
	Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE
Pyknotic Eryhrocyte (%)	0.0	0.3	0.125±0.03	0.0	0.5	0.114±0.04
Mitotic Eryhrocytes (%)	0.0	0.4	0.042±0.03	0.0	0.3	0.021±0.02
Anucleated Erythrocyte (%)	0.0	0.1	0.017±0.01	0.0	0.3	0.079±0.02
Nuclear Shift (%)	0.0	6.4	1.167±0.53	0.2	2.7	0.650±0.18
Elliptical Shape Disorders (%)	0.1	2.0	0.858±0.15	0.1	2.4	0.771±0.14
Vacuolisation (%)	0.0	1.6	0.808±0.16	0.1	3.3	0.893±0.23
Total Erythrocytic Abnormalities (%)	1.20	7.70	3.01±0.55	0.70	4.80	2.52±0.32
IE (‰)*	19	78	48.58±5.63	2	100	21.14±7.36

* Statistically significant differences between species.

When examining the results of interspecies comparison of erythrocytic abnormalities, although there were no statistically significant differences, it was observed that *Natrix natrix* had higher values in all parameters except for anucleated erythrocytes (AE) and vacuolisation (V). Overall, in terms of total erythrocytic abnormalities, it was determined that the *N. natrix* (3.01±0.55%) had higher values than *N. tessellata* (2.52±0.32%). It was reported that cytoplasmic vacuolisation values in a watersnake species (*Homolopsis buccata*) was less than 1% (Salakij et al., 2002) and in a colubrid species as 11.06% (Strunjak-Perovic et al., 2010). In snakes, cytoplasmic vacuolisation can arise spontaneously as an adaptive physiological response (Canfield, 1998) or because of apoptosis induced by some stress factors such as ischemia, hypoxic conditions, and growth factor deprivation (Morris et al., 1984; Mower et al., 1994; Araki et al., 2006; Strunjak-Perovic et al., 2010). Exposure to pesticides or heavy metal pollution may alter erythrocytes shape to become rounded or teardrop-shaped (echinocytes) to compensate for the reduced oxygen content in the water bodies and subsequent cellular abnormalities often lead to cell death (Valbona et al., 2015).

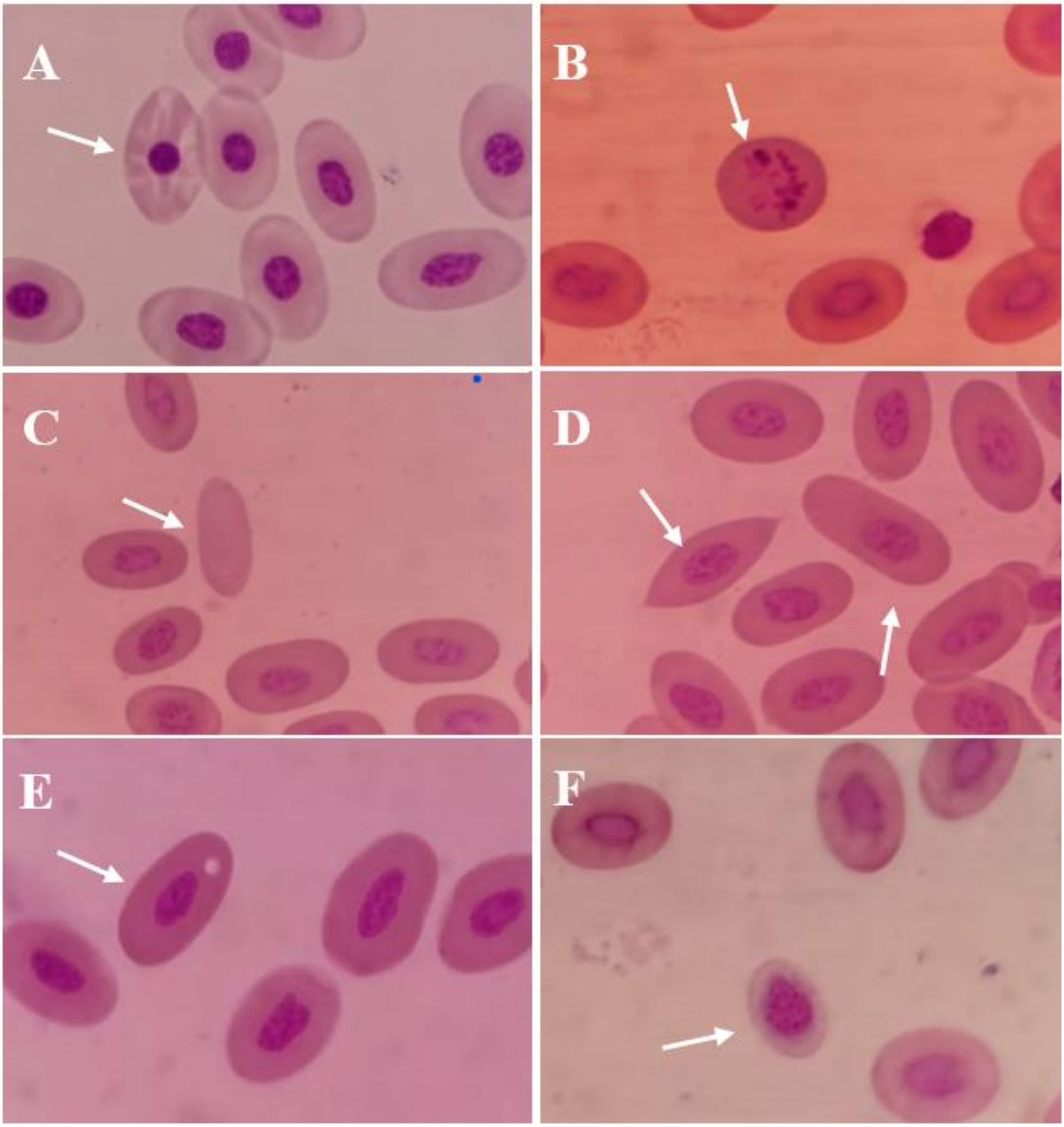

Figure 2. Eryhrocytic abnormalities and immature erythrocytes in *N. natrix* and *N. tessellata* from Biga Stream; Pyknotic Erythrocyte (A), Mitotic Erythrocyte (B), Anucleated Erythrocyte (C), Elliptical Shape Disorders and Nuclear Shift (D), Vacuolisation (E), Immature erythrocyte and mature ones (F).

Shape of the blood cells can change in snakes that have spent a long time in brackish and saline waters (Koleva et al., 2017) such as our study site. Moreover, the increased frequencies of anucleated and mitotic cells may serve as a short-term mechanism to boost oxygen-carrying capacity while pyknotic cells are associated with apoptosis (Barni et al., 2007; Saquib et al., 2012; Peltzer et al., 2013; Pollo et al., 2019).

There was statistically significant difference in the immature erythrocyte index (IE) between the two species. *Natrix natrix* has higher immature erythrocytes (48.58±5.63‰) than *N. tessellata* (21.14±7.36‰). Lisičić et al. (2013) determined that in males of *Vipera ammodytes* distributed in Croatia, the percentage of immature erythrocytes ranged from 0-5%, while in females it ranged from 0-1.24%. In *V. ammodytes* individuals from Türkiye, immature erythrocytes were found as 4.0-8.0% (Baycan et al., 2022). The imbalance between ratio of immature and mature erythrocytes identified in blood samples may suggest the presence of a disease or infection in the individual (Campbell, 2006; Oliveira-Junior et al., 2009; Lisicic et al., 2013). Furthermore, studies conducted on various vertebrate animal groups have also assessed the frequency of immature erythrocytes to support the findings of nuclear abnormalities (Minissi et al., 1996; Pacheco & Santos, 2002; Guilherme et al., 2008).

The frequencies of nuclear abnormalities in erythrocytes were measured in each species captured at the same locality and summarized in Table 2. Our results have shown that the frequency of total nuclear abnormalities was 13.40±2.7% in *Natrix natrix* and 12.68±5.81% in *N. tessellata*. However, there is no statistically significant difference in two species ($p \leq 0.05$). The nuclear abnormalities such as lobbed nuclei, notched nuclei, blebbed nuclei, and binucleated cells were observed in the samples of each species and frequencies were calculated separately (Fig. 3). Analysis of frequencies in peripheral erythrocytes of *N. natrix* and *N. tessellata* from Biga Stream showed that the frequency of nuclear abnormalities such as lobbed, notched, blebbed nucleus was higher than the other abnormalities. *N. natrix* can be found in both aquatic and terrestrial habitats, resulting in more diverse dietary preferences compared to other species (Kornilev et al., 2023). On the other hand, *N. tessellata* spend more time in water bodies (Scali, 2011). *N. natrix* is feeding on amphibians and fish, whereas *N. tessellata* mainly eats fish (Šukalo et al., 2014; Speybroeck et al., 2016; Kornilev et al., 2023). Owing to their habitat preference and dietary habits, *N. natrix* is more likely to be exposed to various pollutants in land as well as water. In previous studies, 10.89% nuclear abnormalities and 0.03% micronucleus frequency were reported from *Hierophis gemonensis* (Strunjak-Perovic et al., 2010). While there was 0.23% micronucleus frequency, 15.71% notched nucleus frequency, 11.63% blebbed nucleus frequency in *N. tessellata* from Vize/Kırklareli, Türkiye; 0.30% micronucleus frequency, 21.93% notched nucleus frequency, 8.85% blebbed nucleus frequency were determined from Kızılcahamam, Ankara, Türkiye (Tok et al., 2014). It was also reported that nuclear abnormality findings of *V. ammodytes* were as 0-0.1% micronucleus, 0.3-0.8% lobbed nucleus, 3.2-4.6% notched nucleus, 2.6-3.9% blebbed nucleus, 0-0.1% binucleate, and 0.3-0.4% kidney shaped nucleus frequency (Baycan et al., 2022). In our study, micronucleus formation was not observed in the samples of each species. Thus, our results suggested that the other nuclear abnormalities should be used to determine the levels of genetic damage in erythrocytes of *Natrix sp*. Erythrocyte nuclear deformities may occur because of the cell detecting an affected region and initiating a process of chromatin repair and elimination (Shimizu et al., 1998; Ergene et al., 2007). The occurrence of micronuclei and other nuclear abnormalities in snakes may fluctuate based on factors such as environmental pollutants (Tok et al., 2014), agricultural activities (Baycan et al., 2022), seasons, characteristics of the species, and gender (Strunjak-Perovic et al., 2010).

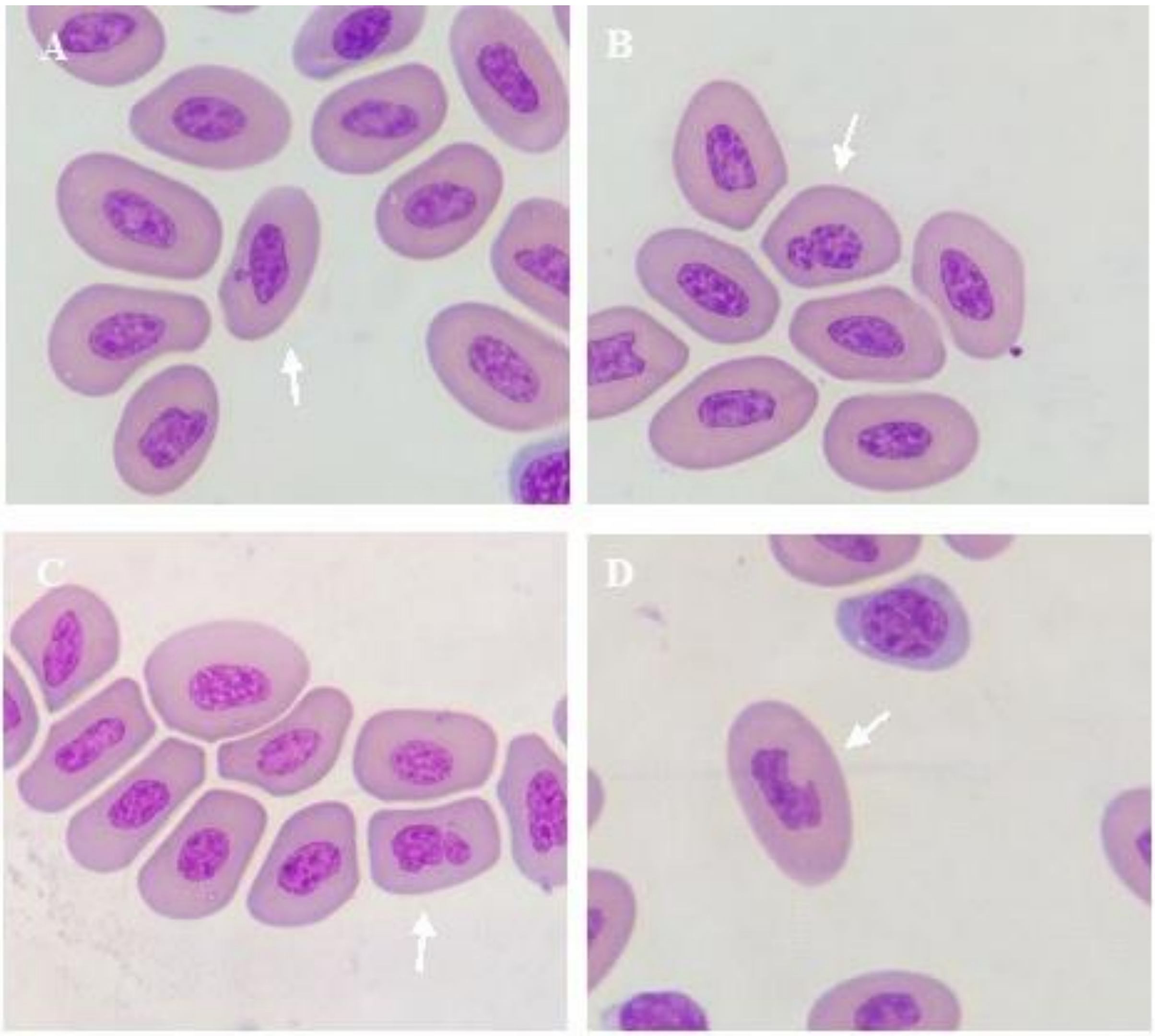

Figure 3. Nuclear abnormalities in *N. natrix and N. tessellata* erythrocytes from Biga Stream; Blebbed nucleus (A), Lobbed nucleus (B), Binucleated cell (C), and Notched nucleus (D)

Table 2. Nuclear abnormalities frequencies (%) in *Natrix natrix* and *N. tessellata* erythrocytes from the Biga Stream.

	MN (%)	Lobbed Nucleus (%)	Notched Nucleus (%)	Blebbed Nucleus (%)	Binucleated cell (%)	Total Nuclear Abnormalities (%)
N. natrix	-	1.35±0.58	3.61±0.39	8.43±3.15	-	13.40±2.7[a]
N. tessellata	-	0.33±0.35	1.60±0.43	10.70±5.56	0.03±0.05	12.68±5.81[a]

*Means with the same letter in the columns are not significantly different at p ≤ 0.05

In conclusion, this study provides fundamental data on general stress indicators such as erythrocytic abnormalities and nuclear abnormalities in two water snake species *Natrix natrix* and *N. tessellata* suggesting that these analyses could be useful for assessing exposure to environmental pollution of reptiles. As a result, there were no statistically significant differences in erythrocytic and nuclear abnormalities between the two *Natrix* species living coexisting sympatrically in the Biga Stream while there was statistically significant difference between these species only in the immature erythrocyte index which was higher in *N. natrix*. Due to the results, it is suggested that *N. natrix* may exhibit more physiological responses to envirenmental stress factors. However, further studies in various polluted areas will be necessary to evaluate the role of different pollutants.

Ethics committee approval: The study was conducted with the permission of Çanakkale Onsekiz Mart University, Animal Experiments Local Ethics Committee (Permit no. 2017/04-16).

Conflict of interest: The authors declare that there is no conflict of interest.

Author Contributions: Conception – N.Ç.; Design – N.Ç.; Supervision: N.Ç., İ.U.; Fund: B.BA., İ.U.; Materials – B.BA., B.BO., İ.U.; Data Collection or Processing: B.BO., B.BA.; Analysis Interpretation – B.BO., B.BA.; Literature Review – N.Ç., İ.U.; Writing – N.Ç., B.BO., B.BA., İ.U.; Critical Review – N.Ç., B.BO., B.BA., İ.U.

References

Akbulut, M., Odabaşı, S.S., Odabaşı, D.A., & Çelik, E.Ş. (2006). Çanakkale İli'nin Önemli İçsuları ve Kirletici Kaynakları. *Su Ürünleri Dergisi*, 23(1), 9-15.

Araki, T., Matsuhiko, H., & Saruta, T. (2006). Annion-exchange blocker enhances cytoplasmic vacuole formation and cell death in serum-deprived mouse kidney epithelial cells in mice. *Cell Biology International*, 30, 93–100. https://doi.org/10.1016/j.cellbi.2005.10.020

Arıkan, H., & Çiçek, K. (2010). Morphology of peripheral blood cells from various species of Turkish herpetofauna. *Acta Herpetologica*, 5, 179–198.

Ballard, B.M., & Cheek, R. (2003). Exotic animal medicine for the veterinary technician. Wiley and Sons / Blackwell Publishing, New York.

Baran, İ., Ilgaz, Ç., Avcı, A., Kumlutaş, Y., & Olgun, K. (2012). Türkiye Amfibi ve Sürüngenleri. Tübitak Popüler Bilim Kitapları, 207, Semih Matbaacılık, Ankara. 204 p.

Barni, S., Boncompagni, E., Grosso, A., Bertone, V., Freitas, I., Fasola, M., & Fenoglio, C. (2007). Evaluation of *Rana* snk *esculenta* blood cell response to chemical stressors in the environment during the larval and adult phases. *Aquatic Toxicology*, 81(1), 45-54. https://doi.org/10.1016/j.aquatox.2006.10.012

Baycan, B., Boran, B., Gül, Ç., & Tosunoğlu, M. (2022). Clinical Hematology of the Nose-Horned Viper, *Vipera ammodytes* (Linnaeus 1758). *Reptiles & Amphibians*, 29(1), 461-469. https://doi.org/10.17161/randa.v29i1.16650

Bolognesi, C., & Hayashi, M. (2011). Micronucleus assay in aquatic animals. *Mutagenesis*, 26, 205-213. https://doi.org/10.1093/mutage/geq073

Campbell, T.W. (2006). Clinical pathology of reptiles, pp 453–470. In: D.R. Mader (ed.), Reptile Medicine and Surgery. Saunders, Philadelphia, PA.

Canfield, P.J. (1998). Comparative cell morphology in the peripheral blood film from exotic and native animals. *Australian Veterinary Journal*, 76(12), 793–800. https://doi.org/10.1111/j.1751-0813.1998.tb12328.x

Carrasco, K., Tilbury, K.L., & Myers, M.S. (1990). Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. *Canadian Journal of Fisheries and Aquatic Sciences*, 47, 2123–2136. https://doi.org/10.1139/f90-237

Erdoğan, D., & Tosunoğlu, M. (2017). Plasma biochemical parameters of *Natrix natrix* (Linnaeus, 1758; Squamata: Natricidae) Population in Çanakkale. *Russian Journal of Herpetology*, 24(1). https://doi.org/10.30906/1026-2296-2017-24-1-35-40

Ergene, S., Cavas, T., Celik, A., Koleli, N., Kaya, F., & Karahan, A. (2007). Monitoring of nuclear abnormalities in peripheral erythrocytes of three fish species from the Goksu Delta (Turkey): genotoxic damage in relation to water pollution. *Ecotoxicology*, 16, 385-391. https://doi.org/10.1007/s10646-007-0142-4

Fenech, M. (2000). The *in vitro* micronucleus technique. *Mutation Research*, 455, 81–95. https://doi.org/10.1016/S0027-5107(00)00065-8

Frenzilli, G., Nigro, M., & Lyons, B.P. (2009). The Comet assay for the evaluation of genotoxic impact in aquatic environments. *Mutation Research/Reviews in Mutation Research*, 681(1), 80-92. https://doi.org/10.1016/j.mrrev.2008.03.001

Guilherme, S., Válega, M., Pereira, M.E., Santos, M.A., & Pacheco, M. (2008). Erythrocytic nuclear abnormalities in wild and caged fish (*Liza aurata*) along an environmental mercury contamination gradient. *Ecotoxicology and Environmental Safety*, 70(3), 411-421. https://doi.org/10.1016/j.ecoenv.2007.08.016

Gül, Ç., Hacıoğlu-Doğru, N., & Tosunoğlu, M. (2018). Impact of Some Hematological and Microbiological Factors on two *Natrix* Species in the Biga Stream (Çanakkale, Turkey). *American Journal of Innovative Research and Applied Sciences*, 6(4), 203-209.

Gül, Ç., Tosunoğlu, M., Bilgin, H., Baycan, B., Boran, B., & Kurtul, D. (2019). Determination of the effect of body size on the hematological parameters in the species of *Natrix tessellata* (Tessellated water snake) distributed in Çanakkale (Turkey), I. International Symposium on Biodiversity Research, Çanakkale, Türkiye, 2-4 Mayıs 2019, pp. 317.

Haskins, D.L., Brown, M.K., Bringolf, R.B., & Tuberville, T.D. (2021). Brown watersnakes (*Nerodia taxispilota*) as bioindicators of mercury contamination in a riverine system. *Science of The Total Environment*, 755, 142545. https://doi.org/10.1016/j.scitotenv.2020.142545.

Heddle, J.A., & Countryman, R.I. (1976). The production of micronuclei from chromosome aberration in irradiated cultures of human lymphocytes. *Mutation Research/Fundamental and Molecular Mechanism of Mutagenesis*, 41, 321-332. https://doi.org/10.1016/0027-5107(76)90105-6

Hojati, V., Faghiri, A., & Shiravi, A. (2012). Diet of the grass snake, *Natrix natrix* (Linnaeus, 1758) (Serpentes: Colubridae), in northern Iran. *Zoology in the Middle East*, 55(1), 132-134. https://doi.org/10.1080/09397140.2012.10648929

IUCN. (2023). The IUCN Red List of Threatened Species. Version 2022-2. https://www.iucnredlist.org

Josende, M. E., Tozetti, A. M., Alalan, M. T., Filho, V.M., Ximenez, S., Junior, F.M.R., & Martins, S.E. (2015). Genotoxic evaluation in two amphibian species from Brazilian subtropical wetlands. *Ecological Indicators*, 49, 83–87. https://doi.org/10.1016/j.ecolind.2014.10.007

Koleva, V., Kornilev, Y., Telenchev, I., Lukanov, S., Hristova, B., & Natchev, N. (2017). Salt tolerance's toll: prolonged exposure to saline water inflicts damage to the blood cells of dice snakes (*Natrix tessellata*). *Web Ecology*, 17(1), 1-7. https://doi.org/10.5194/we-17-1-2017

Kornilev, Y. V., Popgeorgiev, G., Plachiyski, D., Dyugmedzhiev, A., Mladenov, V., Andonov, K., ... & Naumov, B. Distribution of the grass snake (*Natrix natrix*) and dice snake (*N. tessellata*) in Bulgaria. (2023). *Historia Naturalis Bulgarica*, 45(9), 239-254. https://doi.org/10.48027/hnb.45.093

Kousar, S., & Javed, M. (2015). Studies on Induction of Nuclear Abnormalities in Peripheral Blood Erythrocytes of Fish Exposed to Copper. *Turkish Journal of Fisheries and Aquatic Sciences*, 15, 879-886. https://doi.org/10.4194/1303-2712-v15_4_11

Lettoof, D.C., Cornelis, J., Jolly, C.J., Aubret, F., Gagnon, M.M., Hyndman, T.H., ... & Bateman, P.W. (2022). Metal (loid) pollution, not urbanisation nor parasites predicts low body condition in a wetland bioindicator snake. *Environmental Pollution*, 295, 118674. https://doi.org/10.1016/j.envpol.2021.118674

Lisičić, D., Đikić, D., Benković, V., Knežević, A.H., Oršolić, N., & Tadić, Z. (2013). Biochemical and hematological profiles of a wild population of the nosehorned viper *Vipera ammodytes* (Serpentes: Viperidae) during autumn, with a morphological assessment of blood cells. *Zoological Studies*, 52, 1–9. https://doi.org/10.1186/s40409-017-0098-7.

Manolis, S., Webb, G., & Britton, A. (2002). Crocodilians and Other Reptiles: Bioindicators of Pollution.

Marcussi, S., Santos, P.R.S., Menaldo, D., Silveira L., Santos-Filho, N., Mazzi, M., Silva, S., Stábeli, R., Greggi Antunes, L., & Soares, A. (2011). Evaluation of the genotoxicity of *Crotalus durissus terrificus* snake venom and its isolated toxins on human lymphocytes. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 724(1-2), 59-63. https://doi.org/10.1016/j.mrgentox.2011.06.004

Minissi, S., Ciccotti, E., & Rizzoni, M. (1996). Micronucleus test in erythrocytes of *Barbus plebejus* (Teleostei, Pisces) from two natural environments: a bioassay for the in situ detection of mutagens in freshwater. *Mutation Research*, 367, 245–251. https://doi.org/10.1016/S0165-1218(96)90084-1.

Morris, R.G., Hargreaves, A.D., Duvall, E., & Wyllie, A.H. (1984). Hormoneinduced cell death. 2. Surface changes in thymocytes undergoing apoptosis. *American Journal of Pathology*, 115, 426–436

Mower, D.A., Peckham, D.W., Illera, V.A., Fishbaugh, J.K., Stunz, L.L., & Ashman, R.F. (1994). Decreased membrane phospholipids packing and decreased cell size precede DNA cleavage in mature mouse B cell apoptosis. *Journal of Immunology*, 152, 4832–4842. https://doi.org/10.4049/jimmunol.152.10.4832

Napierska, D., Barŝienė, J., Mulkiewicz, E., Podolska, M., & Rybakovas, A. (2009). Biomarker responses in flounder *Platichthys flesus* from the Polish coastal area of the Baltic Sea and applications in biomonitoring. *Ecotoxicology*, 18, 846–859. https://doi.org/10.1007/s10646-009-0328-z

Nascimento, F.F., Pereira, L.G., Geise, L., Bezerra, A.M., & D'Andrea, P.S. (2011). Colonization process of the Brazilian common vesper mouse, *Calomys expulsus* (Cricetidae, Sigmodontinae): a biogeographic hypothesis. *Journal of Heredity*, 102, 260–268. https://doi.org/10.1093/jhered/esr012

O'Connor, J.S., & Dewling, R.T. (1986). Indices of marine degradation: their utility. *Environmental Management*, 10, 335–343. https://doi.org/10.1007/BF01867257

Obiakor, M.O., Okonkwo, J.C., Nnabude, P.C., & Ezeonyejiaku, C.D. (2012). Eco-genotoxicology: Micronucleus assay in fish erythrocytes as in situ aquatic pollution biomarker: a review. *Journal of Animal Science Advances*, 2, 123-133.

Oliveira-Júnior, A.A., Tavares-Dias, M., & Marcon, J.L. (2009). Biochemical and hematological reference ranges for Amazon freshwater turtle, *Podocnemis expansa* (Reptilia: Pelomedusidae), with morphologic assessment of bloodcells. *Research in Veterinary Science*, 86, 146–151. https://doi.org/10.1016/j. rvsc.2008.05.015.

Pacheco, M., & Santos, M.A. (2002). Biotransformation, genotoxic, and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). *Ecotoxicology and Environmental Safety*, 53, 331–347. https://doi.org/10.1016/s0147-6513(02)00017-9.

Paoletti, M. G., & Sommaggio, D. (1996). Biodiversity indicators for sustainability. Assessment of rural landscapes. *Bioindicator Systems for Soil Pollution*, NATO ASI Series 16, 123-140.

Peltzer, P.M., Lajmanovich, R.C., Attademo, A.M., Junges, C.M., Cabagna-Zenklusen, M.C., Repetti, M.R., Sigrist, M.E., & Beldomenico, H. (2013). Effect of exposure to contaminated pond sediments on survival, development, and enzyme and blood biomarkers in veined treefrog (*Trachycephalus typhonius*) tadpoles. *Ecotoxicology Environmental Safety*, 98, 142-151. https://doi.org/10.1016/j.ecoenv.2013.09.010

Pollo, F.E., Grenat, P.R., Otero, M.A., Babini, S., Salas, N.E., & Martino, A.L. (2019). Evaluation in situ of genotoxic and cytotoxic response in the diploid/polyploid complex *Odontophrynus* (Anura: Odontophrynidae) inhabiting agroecosystems. *Chemosphere*, 216, 306-312. https://doi.org/10.1016/j.chemosphere.2018.10.149

Salakij, C., Salakij, J., Suthunmapinunta, P., & Chanhome, L. (2002). Hematology, morphology and ultrastructure of blood cells and blood parasites from puff-faced watersnakes (*Homalopsis buccata*). *Kasetsart Journal* (Nat Sci), 36, 35–43.

Saquib, Q., Al-Khedhairy, A.A., Siddiqui, M.A., Abou-Tarboush, F.M., Azam, A., & Musarrat, J. (2012). Titanium dioxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in human amnion epithelial (WISH) cells. *Toxicology in Vitro*, 26(2), 351-361. https://doi.org/10.1016/j.tiv.2011.12.011

Scali, S. (2011). Ecological comparison of the Dice Snake (*Natrix tessellata*) and the Viperine Snake (*Natrix maura*) in northern Italy. *Mertensiella*, 18, 131-144.

Sharma, R.C., & Rawat, J.S. (2009). Monitoring of aquatic macroinvertebrates as bioindicator for assessing the health of wetlands: a case study in the Central Himalayas, India. *Ecological Indicators*, 9, 118–128. https://doi.org/10.1016/j. ecolind.2008.02.004.

Shimizu, N., Itoh, N., Utiyama, H., & Vahl, G.M. (1998). Selective entrapment of extrachromosomally amplified DNA by nuclear budding and micronucleation during the S-phase. *Journal of Cell Biology*, 140, 1307–1320. https://doi.org/10.1083/jcb.140.6.1307

Siddig, A.A.H., Ellison, A.M., Ochs, A., Villar-Leeman, C., & Lau, M.K. (2016). How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication. *Ecological Indicators*, 60, 223–230. https://doi.org/10.1016/j.ecolind.2015.06.036.

Speybroeck J., Beukema W., Bok B., Van Der Voort J., Velikov I. (2016). Field Guide to the Amphibians and Reptiles of Britain and Europe. Bloomsbury Publishing, London, 432 pp.

Strunjak-Perovic, I., Lisicic, D., Coz-Rakovac, R., Topic Popovic, N., Jadan, M., Benkovic, V. & Tadic, Z. (2010). Evaluation of micronucleus and erythrocytic nuclear abnormalities in Balkan whip snake *Hierophis gemonensis*. *Ecotoxicology*, 19, 1460-1465. https://doi.org/10.1007/s10646-010-0531-y

Šukalo G., Đorđević S., Gvozdenović S., Simović A., Anđelković M., Blagojević V., Tomović L. (2014). Intra- and inter-population variability of food preferences of two *Natrix* species on the Balkan peninsula. *Herpetological Conservation and Biology*, 9(1), 123−136.

Thrall, A., Baker, D.C., Campbell, T.W., DeNicola, D., Feetman, M.J., Lassen, E.D., Rebar, A., & Weiser, G. (2004). Veterinary hematology and clinical chemistry. Blackwell Publishing. Philadephia, USA.

Titenko-Holland, N., Windham, G., Kolachana, P., Reinisch, F., Parvatham, S., Osorio, A.M., & Smith, M.T. (1997). Genotoxicity of malathion in human lymphocytes assessed using the micronucleus assay in vitro and in vivo: a study of malathion-exposed workers. *Mutation Research/Reviews in Mutation Research*, 388(1), 85-95. https://doi.org/10.1016/S1383-5718(96)00140-4

Tok, C. V., Tosunoğlu, M., Gül, Ç., Yiğini, B., Türkakın, M., Saruhan, G., & Kaya, S. (2006). Erythrocyte count and size in some colubrids (Reptilia: Ophidia) from Turkey. *Russian Journal of Herpetology*, 13(2), 97-100. https://doi.org/10.30906/1026-2296-2006-13-2-97-100

Tok, C.V., Hayretdağ, S., Gürkan, M., & Yakın, B.Y. (2014). A preliminary study on micronuclei and nuclear abnormalities in the erythrocytes of some colubrid snakes from Turkey. *Biharean Biologist*, 8, 53–55.

Tosunoğlu, M., Gül, Ç., Yılmaz, N., & Topyıldız, H. (2011). Hematological Reference Intervals of Some Snake Species in Turkey. *Turkish Journal of Zoology*, 35(2), 237-243. https://doi.org/10.3906/zoo-0905-9

Valbona, A., Mihallaq, Q., & Evis, L. (2015). Grass snake, *Natrix sp*, as a potential bioindicator of freshwater ecosystem's health. A case study in Erzeni River, Tirana, Albania. In International CAFE Conference, France.

van der Horst, G., Walton, S., & Hofmeyr, M.D. (2013). Accurate automated quantitative imaging of tortoise erythrocytes using the NIS image analysis system. *Biotechnic & Histochemistry*, 88(5), 242-249. https://doi.org/10.3109/10520295.2013.765594

Yermolenko, S.V., Gasso, V.Y., Hagut, A.M., Hasso, I.A., & Spirina, V.A. (2019). Bioaccumulation of heavy metals in the dice snake *Natrix tessellata* (Reptilia, Colubridae) from ecosystems of different anthropogenic loads in modern conditions of the steppe Dnieper area. *Ecology and Noospherology*, 30(2), 113-117. https://doi.org/10.15421/031919