



ANNOTATIONS OF THE MATERIALS UNDER ARTICLE 65 OF THE REGULATIONS FOR THE DEVELOPMENT OF THE ACADEMIC STAFF OF PLOVDIV UNIVERSITY

by Chief Assist. Prof. Vesela Ilieva Mitkovska, PhD

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regarding the competition for the academic position of **Associate Professor**,
announced in State Gazette no. 98/19.11.2024

by field of higher education 4. Natural Sciences, Mathematics, and Informatics,
professional field 4.3. Biological Sciences (Zoology - Vertebrate Zoology)

I. Fulfillment of the conditions for holding the academic position of Associate Professor according to Article 65 (1) of the Regulations for the Development of the Academic Staff of University of Plovdiv "P. Hilendarski", following the national and additional faculty requirements.

I have been a member of the Department of Zoology since 2003, where I worked as a biologist and assistant professor until my dissertation defense. I obtained my Ph.D. in Zoology in 2014, for which I submitted the original and copies of the diploma with the relevant sets of documents for the competition. In 2015, I took the academic position of Chief Assistant Professor in Vertebrate Zoology. My total experience as a lecturer on a basic contract at the University is 13 years and 9 months, for which I am providing a certificate from the HR Department of University of Plovdiv "Paisii Hilendarski".

For participation in the competition for the academic position of "Associate Professor" in the scientific specialty of Zoology, I submitted a total of 22 scientific papers that were not used in the procedures for the acquisition of the educational and scientific degree "Doctor" and for the academic position of Chief Assistant Professor. Following the minimum national requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria and the Regulations for the Implementation of the same Law, the attached scientific works are classified as follows:

- 21 scientific publications, all in scientific journals classified by quartiles and refereed, and indexed in world-renowned databases of scientific information (Web of Science and Scopus), of which:
 - 6 scientific papers to indicator “B”.4 (Q1 - 2; Q2 - 2; Q3 - 2).
 - 15 scientific papers to indicator “T”.7 - 15 pcs (Q1 - 2 pcs; Q3 - 7 pcs; Q4 - 6 pcs).
- 1 collective monograph (Indicator “T”.8);
- 50 citations in scientific journals referenced and indexed in world-renowned databases of scientific information (Web of Science and Scopus).

Following the additional requirements of the Faculty of Biology, I also submit:

- 2 textbooks (both co-authored);
- 7 successfully defended graduate students in the respective field of the competition;
- participation in 11 research projects and programs;
- participation in faculty administrative activities in committees for accreditation procedures and quality of education, and other administrative activities.

All presented scientific publications are co-authored and in English, generating a total **impact factor of 28.361** and a total **SJR of 8.557**. I am the lead author in nine of the publications.

In Table 1, I indicate the fulfillment of the minimum national requirements with the number of points by groups of scientific-metric indicators for the academic position of Associate Professor in the professional field 4.3 Biological sciences and their respective performance. Their detailed description and fulfillment are presented in the Report on compliance with the minimum national and additional faculty requirements, supplemented by the relevant references and service notes enclosed in the review kits.

Table 1. Summary of the compliance of the points of Chief. Assist. Prof. Vesela Mitkovska, PhD, with the minimum scientific-metric criteria for holding the academic position of Associate Professor in professional field 4.3. Biological Sciences (Zoology – Vertebrate Zoology).

Group Indicators	Contents	Minimum points for associate professor	Candidate Points
“A“	Indicator 1	50	50
“B“	Indicators 3 or 4	100	120 (from indicator 4)
“Г“	The sum of indicators from 5 to 10	200	242 (from indicator 7 и 8)
“Д“	The sum of points in Indicator 11	50	100
	TOTAL NUMBER POINTS:	400	512

II. Annotations of the scientific papers.

1. Indicator “B”.4. Habilitation work – scientific publications in journals that are referenced and indexed in world-renowned databases of scientific information (Web of Science and Scopus) – 6 publications.

№ B.4.1. **Mitkovska, V.**, Dimitrov, H., & Chassovnikarova, T. (2020). Chronic exposure to lead and cadmium pollution results in genomic instability in a model biomonitor species (*Apodemus flavicollis* Melchior, 1834). *Ecotoxicology and Environmental Safety*, 194, 110413. [Q1 (SJR₂₀₂₀=1.377) / IF₂₀₂₀=6.291] <https://doi.org/10.1016/j.ecoenv.2020.110413>

ABSTRACT: Polymetal dust is a common industrial pollutant. While the use of remediation filters and equipment in lead smelters has reduced pollutant emission, surrounding areas remain contaminated due to the long-term transfer of heavy metals along the food chain. Here we assess the mutagenic potential of the lead-zinc smelter near Plovdiv (Bulgaria) situated in an area that has been contaminated with heavy metals for 60 years. We aimed to evaluate the genomic response of the yellow-necked mouse (*A. flavicollis*), a biomonitor species, in three sampling sites along the pollution gradient. Mice from Strandzha Natural Park were used as a negative control. The bioaccumulation rate of two non-essential heavy metals, lead (Pb) and cadmium (Cd), in liver tissues was determined by atomic absorption spectroscopy. Genetic alterations attributable to chronic exposure to trace levels of heavy metals were assessed in different blood cell populations using two independent methods: a micronucleus test was applied to evaluate the clastogenic and aneugenic alterations in erythrocytes, while a comet assay was used to assess DNA instability, as evidenced by single- and double-stranded breaks and alkali-labile sites, in leucocytes. We observed elevated levels of Pb and Cd in livers derived from mice from the impacted area: the mean Pb concentration ($21.38 \pm 8.77 \mu\text{g/g}$) was two-fold higher than the lowest-observed-adverse-effect levels (LOAELs), while the mean Cd concentration ($13.95 \pm 9.79 \mu\text{g/g}$) was extremely close to these levels. The mean levels of Pb and Cd in livers derived from mice from the impacted area were 31-fold and 63-fold higher, respectively, than the levels measured in mice from the control area. The mean frequency of micronuclei was significantly higher (four-fold) than that observed in the control animals. Furthermore, parameters measured by the comet assay, % tail DNA, tail length and tail moment, were significantly higher in the impact area, indicating the degree of genetic instability caused by exposure to heavy metals. In conclusion, this study shows that despite the reported reduction in lead and cadmium emissions in Bulgaria in recent years, *A. flavicollis* individuals inhabiting areas subject to long-term contamination exhibit significant signs of DNA damage.

№ B.4.2. **Mitkovska, V.**, Dimitrov, H., Popgeorgiev, G., & Chassovnikarova, T. (2024). Nuclear abnormalities and DNA damage indicate different genotoxic stress responses of marsh frogs (*Pelophylax ridibundus*, Pallas 1771) to industrial and agricultural water pollution in South Bulgaria. *Environmental Science and Pollution Research*, 1-19. [Q1₂₀₂₃ (SJR₂₀₂₃=1.006) / IF₂₀₂₂=5.8] <https://doi.org/10.1007/s11356-024-35462-5>

ABSTRACT: Amphibians are continuously exposed to pollutants and anthropogenic stressors in their natural habitats, representing a significant challenge to their survival. This study aimed to quantify the extent of DNA damage caused by chronic industrial and agrochemical surface

water pollution in wild populations of the marsh frog (*Pelophylax ridibundus*). The observed genotoxic effects on the marsh frog DNA, manifesting as abnormalities in erythrocyte nuclei, micronuclei, and DNA strand breaks, demonstrate a clear cause-and-effect relationship with surface water parameters, heavy metals, metalloids, and pesticides. The most prevalent nuclear abnormalities observed were notched and blebbed nuclei and nuclear buds, indicative of chromosomal instability. The significant correlation between cadmium, lead, and copper contamination and the increased frequency of DNA breakage in the marsh frogs from the industrial site indicates that heavy metal contamination has a higher genotoxic potential than pesticide contamination. These findings underscore the vulnerability of amphibians inhabiting heavy metal-contaminated wetlands to genotoxic stress due to their lower tolerance to environmental genotoxins. Therefore, using in situ assays to detect erythrocyte nuclear abnormalities and DNA damage in *P. ridibundus* could serve as a reliable indicator of environmental quality and provide early detection of anthropogenic pollution.

№ B.4.3. **Mitkovska, V.**, & Chassovnikarova, T. (2020). Chlorpyrifos levels within permitted limits induce nuclear abnormalities and DNA damage in the erythrocytes of the common carp. *Environmental Science and Pollution Research*, 27(7), 7166-7176. [Q2 (SJR₂₀₂₀=0.845) / IF₂₀₂₀=4.223] <http://doi.org/10.1007/s11356-019-07408-9>

ABSTRACT: The organophosphate pesticide chlorpyrifos (CPF) is defined as a priority pollutant in surface freshwaters according to Directive 2013/39/EU of the European Parliament. The focus of this study was to assess the potential cytotoxic and genotoxic effects of permissible CPF levels on juvenile forms of the common carp. We found that low-level CPF exposure did not induce elevated levels of micronuclei, but significantly increased the frequency of total nuclear abnormalities (NAs) proportional to dose and time; notched, blebbed, lobed and eight-shaped nuclei, nuclear buds, nuclear bridges and binucleated cells were all detected. Decreased frequencies of polychromatic erythrocytes (PCEs) and DNA damage detected by comet assay were also observed, confirming the cytotoxic and genotoxic effects of CPF. Altogether, these data (1) demonstrate that CPF is toxic even at permissible levels, possessing considerable genotoxic and cytotoxic potential in peripheral erythrocytes of exposed fish and (2) validate the assessment of NAs, PCEs and comet assay performance as sensitive biomarkers for the early detection of CPF pollution. These findings can be applied to guide environmental risk assessment and biomonitoring programs.

№ B.4.4. Christova, I., Plyusnina, A., Gladnishka, T., Kalvatchev, N., Trifonova, I., Dimitrov, H., **Mitkovska, V.**, Mohareb, E. & Plyusnin, A. (2015). Detection of Dobrava hantavirus RNA in *Apodemus* mice in Bulgaria. *Journal of Medical Virology*, 87(2), 263-268. [Q2 (SJR₂₀₁₅=1.033) / IF₂₀₁₅=1.998] <https://doi.org/10.1002/jmv.24033>

ABSTRACT: Several Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) in Europe: Dobrava-Belgrade virus (DOBV), Puumala, Saaremaa, Sochi, and Seoul virus. Although HFRS is endemic in Bulgaria, genome sequences of hantaviruses have never been detected in wild rodents. To identify rodent reservoirs, a total of 691 rodents from three endemic regions were trapped in 2011–2012 and screened by TaqMan RT-PCR for detection of hantaviral genomic RNA. Partial small (S) and/or large (L)-segment sequences were recovered from six *Apodemus* mice: five of the species *A. flavicollis* and one *A. agrarius*. Phylogenetic analysis revealed that all recovered sequences belonged to DOBV. On the phylogenetic trees, the novel Bulgarian hantavirus sequences clustered together with sequences of established

previously DOBV variants recovered from Bulgarian HFRS patients and also with variants found in wild rodents trapped in Slovenia, Greece, and Slovakia. One of the novel Bulgarian DOBV S-sequences from *A. agrarius* was related closely to DOBV sequences recovered from *A. flavicollis*, suggesting a spillover of DOBV from its natural host to *A. agrarius* mice. The results of this study confirmed the circulation of DOBV in wild rodents in Bulgaria. The complexity of the epidemiological situation in the Balkans requires further studies of hantaviruses in rodent hosts and human HFRS cases.

№ B.4.5. **Mitkovska, V.**, Dimitrov, H., & Chassovnikarova, T. (2017). *In vivo* genotoxicity and cytotoxicity assessment of allowable concentrations of nickel and lead: Comet assay and nuclear abnormalities in acridine orange stained erythrocytes of common carp (*Cyprinus carpio* L.). *Acta Zoologica Bulgarica, Suppl. 8*, 47-56. [Q3 (SJR₂₀₁₇=0.217) / IF₂₀₁₇=0.369]
<https://www.acta-zoologica-bulgarica.eu/downloads/acta-zoologica-bulgarica/2017/supplement-8-47-56.pdf>

ABSTRACT: The aim of the present study was to assess the possibility of *in vivo* genotoxicity by nickel and lead concentrations considered safe by regulatory agencies. In order to evaluate their genotoxic and cytotoxic potential, young specimens of the common carp (*Cyprinus carpio* L.) were exposed for 72 h in laboratory conditions to different concentrations of Ni and Pb, considered as annual average according to Directive 2008/105/EC. The alkaline comet assay in circulating erythrocytes was applied and blood smears stained with acridine orange were tested for the presence of micronuclei and other nuclear abnormalities. The comet parameters % tail DNA and tail moment indicated statistically significant genotoxic damage for both metals at 100%, 75% and 50% of the permissible concentrations, as compared to controls. Increased levels of micronuclei were not observed in carp erythrocytes, but we found a significant increase in other nuclear abnormalities - notched, blebbed, lobed, eight-shaped nuclei and nuclear buds. This demonstrated evident cytotoxic effects and confirmed the use of nuclear abnormalities as an effective biomarker. The obtained results confirmed the genotoxic and cytotoxic effects of Ni and Pb, even at low permissible levels, and illustrated the need for additional investigations in order to reduce the allowable concentrations of heavy metals in water basins.

№ B.4.6. **Mitkovska, V.**, Dimitrov, H., Kunchev, A., & Chassovnikarova, T. (2020). Micronucleus frequency in rodents with blood parasites. *Acta Zoologica Bulgarica, Suppl. 15*, 33-41. [Q3 (SJR₂₀₂₀=0.237) / IF₂₀₂₀=0.448]
https://www.acta-zoologica-bulgarica.eu/Suppl_15_07.pdf

ABSTRACT: Some parasites cause a genotoxic effect and induce micronuclei in cells of their rodent hosts. The rodents are frequently used for assessment of environmental pollution; therefore, it is crucial to know whether the genetic damage is due to xenobiotics presenting in their environment or is a result of parasite infection. The purpose of this study was to assess the potential link between blood parasites and micronucleus frequencies in erythrocytes of wild rodent populations from anthropogenic impacted and background regions. Individuals of Macedonian mouse *Mus macedonicus* Petrov & Ružić, 1983, yellow-necked mouse *Apodemus flavicollis* (Melchior, 1834) and common vole *Microtus arvalis* (Pallas, 1778) from heavy metal contaminated region and individuals of striped field mouse *Apodemus agrarius* (Pallas, 1771) and *A. flavicollis* from area with low anthropogenic impact have been studied. In all *Apodemus*

populations, we registered non-identified bacterial infections with *Bartonella* spp., in *M. arvalis* – infections with *Babesia microti*-like piroplasms and *Bartonella* sp., and in *M. macedonicus* – infection with trypanosomes (*Trypanosoma musculi*) and *Bartonella* sp. No statistically significant difference ($p \geq 0.05$) between the average micronucleus frequency of the infected and non-infected animals inhabiting both polluted and unpolluted regions has been proven. The results show that the observed blood parasites cannot be associated with the induction of micronuclei in erythrocytes of the studied rodents. This provides justification to include rodents with the recorded protozoan and bacterial infections in biomonitoring studies for assessment of genotoxicity in contaminated areas.

2. Indicator “Г”.7. – Scientific publication in journals referenced and indexed in world-renowned databases of scientific information (Web of Science and Scopus), outside the habilitation thesis – 15 publications.

№ Г.7.1. **Mitkovska, V.**, Chassovnikarova, T., Vasileva, P., Stoyanov, I., Petrov, P., Petkov, N., & Ivanova, E.N. (2025). Sperm comet assay as a novel tool in assessing genotoxicity in high-mortality honey bee (*Apis mellifera*) populations. *Apidologie*, 56, 13. [Q1 (SJR₂₀₂₃=0.727) / IF₂₀₂₃=2.4] <https://doi.org/10.1007/s13592-024-01137-w>

ABSTRACT: The recent increase in mortality rates amongst honey bee colonies is a cause for concern. Assessing DNA damage in reproductive cells is crucial for species survival. This study aims to evaluate the potential of the sperm comet assay as a tool for in situ assessment of the genotoxic impact on honey bee populations with established high mortality rates. Previous studies have identified the presence of pesticide residues in bees and food stocks within the hives, indicating the existence of genotoxic agents in the localities under investigation. The values of comet assay parameters, namely Tail Intensity (TI%) and Olive Tail Moment (OTM, μm), scored in the sperm cells, increased following the mortality trend of the honey bee populations under study, providing evidence of significant DNA damage occurring during spermatogenesis. The alkaline comet assay in hemolymph confirmed the genotoxic effects observed in the sperm comet assay. The current study demonstrated for the first time that the sperm comet assay could serve as a reliable novel method for assessing genotoxicity in *A. mellifera*.

№ Г.7.2. Georgieva, E., Antal, L., Stoyanova, S., Arnaudova, D., Velcheva, I., Iliev, I., Vasileva, T., Bivolarski, V., **Mitkovska, V.**, Chassovnikarova, T., Todorova, B., Uzochukwubg, I.E., Nyeste, K. & Yancheva, V. (2022). Biomarkers for pollution in caged mussels from three reservoirs in Bulgaria: A pilot study. *Heliyon*, 8(3). p.e09069. eISSN 2405-8440. [Q1 (SJR₂₀₂₂=0.609) / IF₂₀₂₂=4.0] <https://doi.org/10.1016/j.heliyon.2022.e09069>

ABSTRACT: The mussel-watch concept was firstly proposed in 1975, which was later adopted by several international monitoring programs worldwide. However, for the very first time, a field experiment with caged mussels was performed in three reservoirs in Bulgaria to follow the harmful effects of sub-chronic pollution (30 days) of metals, trace, and macro-elements, as well as some organic toxicants, such as polybrominated diphenyl ethers and chlorinated paraffins. Therefore, we studied the biometric indices, histochemical lesions in the gills, biochemical changes in the digestive glands (antioxidant defense enzymes, such as catalase, glutathione reductase, and glutathione peroxidase; metabolic enzymes, such as lactate

dehydrogenase, alanine aminotransferase, and aspartate aminotransferase, and the neurotransmitter cholinesterase), in addition to the DNA damage in the Chinese pond mussel, *Sinanodonta woodiana* (Lea, 1834) in Kardzhali, Studen Kladenets and Zhrebchevo reservoirs in Bulgaria. Significant correlation trends between the pollution levels, which we reported before, and the biomarker responses were established in the current paper. Overall, we found that both tested organs were susceptible to pollution-induced oxidative stress. The different alterations in the selected biomarkers in the caged mussels compared to the reference group were linked to the different kinds and levels of water pollution in the reservoirs, and also to the simultaneously conducted bioaccumulation studies.

№ Г.7.3. Chassovnikarova, T., Atanassov, N., Christova, I., Dimitrov, H., **Mitkovska, V.**, Trifonova, I., Gladnishka, T., Kalvatchev, N., & Mohareb, E. (2013). Hantavirus infections in host populations of Yellow-necked and Field mice (Rodentia: Muridae) in South Bulgaria. *Acta Zoologica Bulgarica*, 65(3), 397-402. [Q3 (SJR₂₀₁₃=0.273) / IF₂₀₁₃=0.357] <https://www.acta-zoologica-bulgarica.eu/downloads/acta-zoologica-bulgarica/2013/65-3-397-402.pdf>

ABSTRACT: The wood and field mice of the genus *Apodemus* are natural reservoirs of hantaviruses: *Dobrava*, *Puumala* and *Saarema* virus types. In the last few years, cases of hemorrhagic fever with renal syndrome were reported from different regions in Bulgaria, but the factors, influencing hantavirus transmission within host populations have not been well investigated. Sensitive molecular genetic methods (real time PCR) for detection of hantaviruses in wood and field mice, captured in endemic for the virus region in Southern Bulgaria, were used. *Dobrava* virus type (DOBV) was genetically determined in two *Apodemus* species: *Apodemus flavicollis* (Melchior, 1834) and *Apodemus agrarius* (Pallas, 1771). The overall DOBV prevalence was 7.7% for *A. flavicollis* and 1.43% for *A. agrarius*. A number of factors, influencing the association of DOBV infections in yellow-necked wood and field mice, were evaluated. The logistic regression model showed four factors in the model outcome: species, age, gender and reproductive status. This analysis highlights the importance of population demography in the successful circulation of hantaviruses. The overwintered reproductively active males of yellow-necked mice appear critical to the success of DOBV circulation and persistence within host populations in South Bulgaria.

№ Г.7.4. Dimitrov, H., **Mitkovska, V.**, Koleva, P., & Chassovnikarova, T. (2015). Genotoxicity biomonitoring of anthropogenic pollution in rice fields using the micronucleus test in striped field mouse (*Apodemus agrarius* Pallas, 1771). *Problems of Infectious and Parasitic Diseases*, 43(2), 36-42. [Q3 (SJR₂₀₁₅=0.188)] https://www.researchgate.net/publication/298712693_Genotoxicity_biomonitoring_of_anthropogenic_pollution_in_rice_fields_using_the_micronucleus_test_in_striped_field_mouse_Apodemus_agrarius_pallas_1771#fullTextFileContent

ABSTRACT: Zoomonitoring of small mammal populations, exposed to potential mutagens, can provide an early detection system for the initiation of cell dysregulation. The striped field mouse (*Apodemus agrarius* Pallas, 1771) is an appropriate zoomonitor species suitable for genotoxicological research, especially due to its wide distribution, r-type reproductive strategy, relatively small home range, high trophic chain position and metabolic rate. The present study was carried out in differently polluted areas. The striped field mice were collected in the rice fields located near Plovdiv (Southern Bulgaria) and in background region of Strandzha Nature Park (Southeastern Bulgaria). The rice fields are exposed to different anthropogenic pollutants

like heavy metals and polycyclic aromatic hydrocarbons, due to the nearby located highway and the use of various fertilizers in agricultural practices. The results showed that anthropogenic pollution in rice fields induces DNA and chromosomal lesions in striped field mouse's cells, which was well demonstrated by the micronucleus assay. The mean frequency of micronuclei in the individuals from the rice fields was significantly higher compared to the mean frequency of the individuals from the background region of Strandzha Nature Park. This proves the existence of geno- and cytotoxic effect in the region of the paddies. The micronucleus assay showed no gender differences. The statistically significant differences in mean frequencies of micronuclei in striped field mice both from the impact and from the background area demonstrated the good genomic sensitivity of the species against anthropogenic pollution. The obtained results confirm the importance of *Apodemus agrarius* as a zoomonitor species for biomonitoring studies in the species' characteristic habitats – wetlands.

№ Г.7.5. Dimitrov, H., **Mitkovska, V.**, & Chassovnikarova, T. (2015). *Trypanosoma* infection in Mediterranean mouse (*Mus macedonicus* Petrov & Ružić, 1983) in Bulgaria. *Problems of Infectious and Parasitic Diseases*, 43(2), 26-29. [Q3 (SJR₂₀₁₅=0.188)]
https://www.researchgate.net/publication/298712963_Trypanosoma_infection_in_mediterranean_mouse_Mus_macedonicus_Petrov_Ruzic_1983_in_Bulgaria#fullTextFileContent

ABSTRACT: *Trypanosoma musculi* is a non-pathogenic stercorarian trypanosome which is infective only to mice. The present study reveals a *Trypanosoma* infection in Mediterranean mouse (*Mus macedonicus* Petrov & Ružić, 1983) from the rice fields in Plovdiv region, Bulgaria. The average established prevalence of the parasite in *Mus macedonicus* was 17.1% with higher infection rate in male (21.1%) compared to female (12.5%) species. All trypanomastigotes exhibited morphological features typical of the subgenus *Herpetosoma* (*Stercoraria* section) to which *T. lewisi*-like parasites belong. These features included: size of approximately $25 \pm 5 \mu\text{m}$, free flagellum, characteristic “C shape”, visible undulating membrane, oval-shaped subterminal kinetoplast and a nucleus at the anterior end. The characteristic morphology and the presence of infection only in individuals of *Mus macedonicus* allow us to make an evidence-based assumption that the observed parasite is *Trypanosoma musculi*. Future characterisation should include molecular methods to confirm the registered species.

№ Г.7.6. Chassovnikarova, T., Atanasov, N., Dimitrov, H., & **Mitkovska, V.** (2017). Karyotypic characterization of the Harvest mouse *Micromys minutus* (Pallas, 1771) (Rodentia: Muridae) from Upper Thracian Valley, South Bulgaria. *Acta Zoologica Bulgarica*, Suppl. 8, 57-60. [Q3 (SJR₂₀₁₇=0.217) / IF₂₀₁₇=0.369] <https://www.acta-zoologica-bulgarica.eu/downloads/acta-zoologica-bulgarica/2017/supplement-8-57-60.pdf>

ABSTRACT: This study presents the first cytogenetical studies of the harvest mouse *Micromys minutus* (Pallas, 1771) in Bulgaria. A population of harvest mice from the rice fields near Plovdiv in the Bulgarian part of the Thracian Valley was examined. Five individuals (3 males and 2 females) were cytogenetically analyzed. The diploid chromosome number was found to be $2n = 68$. Autosomes consisted of 1 pair of large and 1 pair of small metacentric chromosomes, 2 pairs of middle-sized submetacentric chromosomes, 28 pairs of subtelocentric chromosomes and 1 pair of acrocentric chromosomes. The X chromosome was the largest subtelocentric chromosome, whereas the Y chromosome was found to be the smallest acrocentric chromosome. The FN of the chromosomes was estimated to be 133, as the NFa was 130. All

chromosomes, except the largest metacentric pair, exhibited well-expressed heterochromatin blocks in the centromeric regions, which also extended into the short arms of the most bi-armed chromosomes. Block of centromeric heterochromatin was also found on the X chromosome, while the Y chromosome appeared to be entirely heterochromatic.

№ Г.7.7. Vlasseva, A., Chassovnikarova, T., **Mitkovska, V.**, & Dimitrov, H. (2020). Compensatory increase of the reproductive capacity of the red fox *Vulpes vulpes* (L., 1758) in sympatric coexistence with the golden jackal *Canis aureus* L., 1758. *Acta Zoologica Bulgarica, Suppl. 15*, 217-222. [Q3 (SJR₂₀₂₀=0.237) / IF₂₀₂₀=0.448] https://www.acta-zoologica-bulgarica.eu/Suppl_15_39.pdf

ABSTRACT: Main reproductive parameters of the most common mesopredators in Bulgaria, golden jackal and red fox, were studied in two regions (Thracian Lowland and South Dobrudzha) where these two species occur in sympatry. The reproductive activity and the average age of the first birth were calculated in both species. Major reproductive characteristics such as gross reproductive rate, net reproductive rate, generation time and the internal rate of population growth were calculated through a life table based on female individuals. The results demonstrated growing populations of red fox and golden jackal in Bulgaria. The testicular weight (g) of the male individuals was measured and the gonadosomatic index was calculated. The breeding percentage of the studied females was 41% for the jackals and 65% for the red foxes. The maximum number of placental scars in jackals was 9 and in foxes was 8. The minimum number of placental scars in jackals was 2 and in foxes 4. The ratio between net reproductive speed and the time between two successive generations was greater for the fox (40.98) than for the jackal (25.16). The gonadosomatic index was lower for the jackal (0.08) than for the fox (0.19), which is a criterion for a higher degree of monogamy for the jackal. The reproductive parameters studied in both species showed a compensatory increase in the reproductive capacity of the red fox in conditions of sympatry with golden jackals.

№ Г.7.8. Dimitrov, H., Kunchev, A., Markov, G., Chassovnikarova, T., & **Mitkovska, V.** (2020). Haematology data of Striped mouse *Apodemus agrarius* (Pallas, 1771) (Rodentia: Muridae) from Strandzha Mountains, SE Bulgaria: Indicators of environmental status. *Acta Zoologica Bulgarica, Suppl. 15*, 199-203. [Q3 (SJR₂₀₂₀= 0.237) / IF₂₀₂₀=0.448] https://www.acta-zoologica-bulgarica.eu/Suppl_15_36.pdf

ABSTRACT: Haematology parameters are important indicators for the status of both individuals and populations of wild small mammals that are affected by toxicants, diseases and other natural or anthropogenic harmful factors. We report for the first time the values of the following haematological parameters: number of erythrocytes [10¹²/l]; HGB – haemoglobin [g/l]; HCT – haematocrit [%]; MCV – mean corpuscular volume of erythrocytes [fl]; MCH – mean corpuscular haemoglobin of erythrocytes [pg]; MCHC – mean corpuscular haemoglobin concentration [g/l]; WBC – number of leukocytes [10⁹/l]; LYM – number of lymphocytes [10⁹/l]; MID – number of middle cells [10⁹/l]; GRA – number of granulocytes [10⁹/l]; LY – lymphocytes, measured as percentage of total leukocytes [%]; MI – middle cells, measured as percentage of total leukocytes [%]; GR – granulocytes, measured as percentage of total leukocytes [%]; PLT – number of platelets [10⁹/l]; MPV – platelet volume [fl]. These parameters were obtained from adult male and female individuals of striped mouse *Apodemus agrarius*. The mice were collected from the Strandzha Mts. in Bulgaria. The statistical analysis of the established haematological parameters has defined the baseline norm of variation of

values described by $\pm 95\%$ confidence interval. They can be used for the evaluation of the physiological condition of the individuals of the striped mouse as bioindicative markers for evaluating the quality of the environment.

№ Г.7.9. Koteva, E., Dimitrov, H., **Mitkovska, V.**, & Chassovnikarova, T. (2020). Rodent control in urban industrial areas: from research to action. *Acta Zoologica Bulgarica, Suppl. 15*, 211-216. [Q3 (SJR₂₀₂₀=0.237) / IF₂₀₂₀=0.448] https://www.acta-zoologica-bulgarica.eu/Suppl_15_38.pdf

ABSTRACT: Pest control measures have an increasing importance due to the rising economic initiatives and urbanization resulting in a growing number of industries facing pest problems. The use of rodenticides is a very common method in rodent control but the bait consumption depends on the environmental alterations in different industry areas. The aim of the present study was to monitor the control of rodents in facilities belonging to various food industries using rodenticide baits and to determine the factors that could directly influence the bait consumption. In the city of Plovdiv and its region, Bulgaria, 89 bait stations were monitored in different industrial sites: glass factory, croissant factory and dairy. The bait consumption was studied using the electronic monitoring *PestScan* method. The results showed that factors such as industry type, the construction and technical implementation of the buildings, the location of the site and the type and formulation of the rodenticide used influence the decision for the exact bait disposal. Understanding environmental conditions influencing the bait consumption could help to plan the pest control for specific sites by matching with specific strategies.

№ Г.7.10. Dimitrov, H., **Mitkovska, V.**, Tzekov, V., & Chassovnikarova, T. (2016). Bioaccumulation of cadmium and lead in rodent species from the region of lead-zinc smelting factory – Plovdiv (South Bulgaria). *Ecologia Balkanica*, 8(1), 9-18. [Q4 (SJR₂₀₁₆=0.123) / WoS] http://web.uni-plovdiv.bg/mollov/EB/2016_vol8_iss1/009-018_eb.16115.pdf

ABSTRACT: The levels of the toxic metals, Cd and Pb, were measured in liver of yellow-necked mouse (*Apodemus flavicollis* Melchior, 1834), Mediterranean mouse (*Mus macedonicus* Petrov & Ružić, 1983) and common vole (*Microtus arvalis* Pallas, 1778) from the vicinity of Plovdiv (South Bulgaria), where the lead-zinc smelting factory is the main source of pollution. The study was carried out at three sites located along a pollution gradient. An unpolluted region, the Strandzha Natural Park was used as a background region. MANOVA analysis revealed significant differences by species ($F=9.61$, $p=0.003$), site ($F=24.12$, $p=0.0001$) and exposure ($F=3.79$, $P=0.013$) effects. Significant increase of Pb and Cd bioaccumulation was found along the pollution gradient. Cd and Pb mean concentrations were highest at the site closest to the smelter and decreased with increasing the distance from them. The bioaccumulation of Pb was significant highest in the individuals of the yellow-necked mouse, followed by Mediterranean mice and common voles, whereas the common voles accumulated more Cd in comparison with the yellow-necked-, and Mediterranean mice. However, there is little evidence of adverse cadmium-mediated effects in yellow-necked- and Mediterranean mice and this species may be tolerant to Cd exposure. High Cd concentrations in body organs may simply reflect an ability to store the metal in a nontoxic, metallothionein-bound state. Liver Pb and Cd concentration did not differ significantly among sexes.

№ Г.7.11. **Mitkovska, V.**, Dimitrov, H., & Chassovnikarova, T. (2018). Induction of erythrocytic nuclear abnormalities by permitted concentration of cadmium in common carp (*Cyprinus carpio* L.). *Ecologia Balkanica*, 10(2), 235-247. eISSN:1314-0213 [Q4 (SJR₂₀₁₈=0.103 / WoS] http://web.uni-plovdiv.bg/mollov/EB/2018_vol10_iss2/235-247_eb.18125.pdf

ABSTRACT: Abstract. The induction of micronuclei and other nuclear abnormalities (NAs) - nuclear buds, nucleoplasmic bridges, binucleated cells, as well as notched, blebbed, lobed and eight-shaped nuclei - was analyzed in peripheral blood erythrocytes of common carp (*Cyprinus carpio* L.) treated with cadmium (Cd) at legally allowed concentration. Young specimens of the common carp were exposed to the lowest of the four permitted concentrations of Cd (0.45 µg/L) in surface waters, according to Directive 2013/39/EU, for 72 h and 144 h in laboratory conditions. Blood smears were stained with acridine orange and tested for the presence of micronuclei and other NAs. We report that Cd exposure for 72 h resulted in a significantly increased number of blebbed and lobed nuclei and eight-shaped nuclei, as well as an increase in the frequency of Total NAs. Cd exposure for 144 h resulted in an increase in the number of cells with notched nuclei and nucleoplasmic bridges, as well as an increased occurrence of binucleated cells. Our results highlight the cytotoxic and genotoxic effects of Cd, even at low permissible levels, and confirm the use of NAs as an effective biomarker. The elevated levels of NAs observed after just 144 h of exposure to Cd highlight the need for further research. Once confirmed, changes in national and EU legislation of permitted concentrations of Cd in surface waters might be required.

№ Г.7.12. **Mitkovska, V.**, Dimitrov, H., & Chassovnikarova, T. (2021). Chronic exposure to heavy metals induces nuclear abnormalities and micronuclei in erythrocytes of marsh frog (*Pelophylax ridibundus* Pallas, 1771). *Ecologia Balkanica*, Special Edition 4, 97-108. [Q4 (SJR₂₀₂₁=0.137) / WoS] http://web.uni-plovdiv.bg/mollov/EB/2021_SE4/097-108_eb21SE410.pdf

ABSTRACT: Amphibians have big potential as bioindicators based on their combined life cycle as aquatic and terrestrial form. They can play the role of prey or predator, making them a key element in toxic substances transfer between aquatic and terrestrial habitats. The nuclear abnormalities (NAs) in amphibians' erythrocytes in recent years have been used as a successful biomarker for anthropogenic pollution. The NAs including micronuclei in erythrocytes of the marsh frog (*P. ridibundus*) have been studied to assess the cytotoxic and genotoxic effect in heavy metal polluted area *in situ*. Here we assess the cyto- and genotoxic potential of the polluted waters (Chaya River) close to the lead-zinc smelter near Plovdiv (Bulgaria) situated in an area that has been contaminated with heavy metals for 60 years. Frogs from Strandzha Natural Park were used as a negative control. Peripheral blood smears have been dyed with acridine orange. NAs of the following types: notched nuclei, nuclear buds and blebbed nuclei have shown the highest frequency. There is no sexual dependence in the formation of different types of NAs. The significant differences ($P \leq 0.0001$) in the mean Total NAs (‰) in erythrocytes of marsh frogs from the polluted area compared to the total NAs from the background region "Strandzha" NP demonstrate the presence of *in vivo* active cytotoxic and genotoxic agents in the impacted area. The obtained results for NAs in erythrocytes of *P. ridibundus* are evidence for successful application of NAs as a biomarker in amphibians for the purpose of biomonitoring.

№ Г.7.13. **Mitkovska, V.**, Dimitrov, H., & Chassovnikarova, T. (2021). Occurrence of haemoparasites of the genus *Hepatozoon* (Adeleorina: Hepatozoidae) in the marsh frog (*Pelophylax ridibundus* Pallas, 1771) in Bulgaria. *Ecologia Balkanica*, Special Edition 4, 109-116. [Q4 (SJR₂₀₂₁=0.137) / WoS]

http://web.uni-plovdiv.bg/mollov/EB/2021_SE4/109-116_eb21SE411.pdf

ABSTRACT: The distribution of species of *Hepatozoon* from anurans, specifically frogs, and their host-parasite relationships are of great interest. Due to the specifics of the ontogeny of frogs, they form a link between aquatic and terrestrial ecosystems. These amphibians are important in food chains, ensuring the normal functioning of biocenosis. In this study, we present data on the presence of *Hepatozoon* sp. in the marsh frog, *Pelophylax ridibundus* (Pallas, 1771) for the first time in Bulgaria. The blood smears of 137 individuals were investigated by fluorescence microscopy after staining with acridine orange. In three of five studied localities, the presence of apicomplexan haemoparasite from the genus *Hepatozoon* was revealed. Prevalence and parasitaemia values were different in frogs populations inhabiting the Chaya River (27.5% and 11.0%, respectively), Tsalapitsa Rice Fields (6.4% and 9.9%, respectively) and nature wetland Zlato pole (10.0% and 8.1%). It was found that the morphology and morphometric parameters of the parasite gamonts are closest to *Hepatozoon magna*, but molecular tools are required to confirm the genus and species determination. Our findings revealed that acridine orange is appropriate dye for detecting haemoparasites of the genus *Hepatozoon*.

№ Г.7.14. Christova, I., Trifonova, I., Panayotova, E., Dimitrov, H., Gladnishka, T., **Mitkovska, V.**, Taseva, E., & Gergova, I. (2021). Hantaviruses in small mammals in two regions in Bulgaria. *Acta Zoologica Bulgarica*, 73(1), 119-123. [Q4 (SJR₂₀₂₁= 0.213) / IF₂₀₂₁=0.362]

<https://acta-zoologica-bulgarica.eu/2021/002419.pdf>

ABSTRACT: Specific antibodies against Puumala and Dobrava-Belgrade orthohantaviruses were examined in small mammals in Bulgaria for the first time. Seroprevalence rate in *Apodemus flavicollis* was 10.3%, in *Apodemus agrarius* - 14.5%, in *Myodes glareolus* - 12.7% and in *Microtus arvalis* - 11.1%. In Smolyan Region, seroprevalence rates of rodents were 5.9% in *M. glareolus* for Puumala hantavirus in the town of Smolyan and 20% of *A. flavicollis* for Dobrava hantavirus in the village of Smilyan. In Pazardzhik Region, infection rates in rodents were much higher. Antibodies against hantaviruses were found in 10.6% and hantavirus RNA was detected in 6.9% of rodents. One *M. glareolus* from the village of Ravnogor was positive for Puumala hantavirus antibodies and, at the same time, Puumala hantavirus RNA was confirmed. Results of the serosurvey on rodents revealed various rates of infection with hantaviruses in different localities and identified the so called “hot spots”, where the risk for humans is significant: Smolyan, Smilyan, Batak, Velingrad and Peshtera. These findings suggest real risks for humans and are important steps to prevent these severe viral hemorrhagic fevers.

№ Г.7.15. Chassovnikarova, T., Dimitrov, H. & **Mitkovska, V.** (2024). Cytogenetic characteristics of *Apodemus epimelas* (Nehring, 1901) (Rodentia: Muridae) from Bulgaria: constitutive heterochromatin and nucleolar organizer regions distribution. *Acta Zoologica Bulgarica*, Suppl. 20, 53-60. [Q4 (SJR₂₀₂₃=0.216) / IF₂₀₂₃=0.4]
https://www.acta-zoologica-bulgarica.eu/2024/Suppl_20_07.pdf

ABSTRACT: The taxonomic status of the western broad-toothed mouse, *Apodemus epimelas* (Nehring, 1901), was established in 2005 based on variations in morphological and molecular genetic data. Previously, it was considered part of the eastern broad-toothed mouse, *Apodemus mystacinus* (Danford & Alston, 1877). This species is endemic to the western and southern Balkans, including Bulgarian populations along the Struma River. The distribution of the constitutive heterochromatin and the nucleolar organiser regions was examined using C- and NOR banding techniques. The results of this study show that the species has a diploid chromosome number of $2n=48$, with $FN=52$, consisting mainly of acrocentric chromosomes and only two small metacentric autosomal pairs. The distribution and extent of C-bands at pericentromeric regions varied among chromosome pairs and primarily appeared at centromeric positions of the autosomes. The X chromosome was consistently the second largest acrocentric chromosome with distinct intercalated C-bands, while the Y chromosome was present as a small, entirely heterochromatic acrocentric chromosome. Here, we present the distribution of nucleolar organiser regions (NORs) in six pairs of autosomes for the first time. The centromere heterochromatin and NORs mixed (centromeric and telomeric) locations characterised the reported species' cytotype, providing valuable insights into the cytogenetic characteristics of this species.

3. Indicator "I".8. Published chapter of a book or collective monograph

№ Г.8.1. Michailova, P., Chassovnikarova, T., Ilkova, J., Grozeva, S., Chobanov, D., Warchałowska-Śliwa, E., Simov, N., Atanassov, N., **Mitkovska, V.**, Dimitrov, H. (2013). Genome biomarker test system for environmental risk assessment. Pensoft Publishers, Sofia-Moscow, 135 p., ISBN 978-954-642-708-3

ABSTRACT: Genome biomarkers are the basis for fast, comprehensive and effective monitoring system, taking into account the genome response of the natural populations of model plant and animal species to the environmental changes. Today they are widely used in the assessment of the environmental pollution impact on the biodiversity in the water and terrestrial ecosystems. The present study provides cytogenetic and molecular biomarkers considering their theoretical and applied significance for environmental risk assessment in natural populations of invertebrate and vertebrate zoomonitoring species. The observed sensitivity to the environmental stress agents differs for the examined species. Well distinguished molecular and cytogenetic markers were found only in the zoomonitoring species of the families Chironomidae (Diptera), Muridae and Arvicolidae (Mammalia, Rodentia). These allow the creation of an integrated Genome Biomarker Test System (GBTS) for risk assessment of different pollutants, affecting the human health and the environment. The results received could be used as a basis for development of a fast and effective method for environmental risk assessment for the purposes of the National system for monitoring in Bulgaria.