REVIEW

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of the materials submitted for participation in a competition for the academic position of "Associate Professor" at the Plovdiv University "P. Hilendarski" in field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field .4.3 Biological Sciences, area, Zoology-Zoology of Vertebrates.

In the competition for "Assoc.prof.", announced in the newspaper, no. 98 of 19.11.2024 and on the Internet page of Plovdiv University "P. Hilendarski" for the needs of the Department of Zoology, Faculty of Biology, as a candidate participates Ch. assistant Dr. Vessela Ilieva Mitkovska from the Department of Zoology, Faculty of Biology at Plovdiv University "P. Hilendarski ".

General presentation of the received materials

A list of scientific papers, a list of citations, a reference for auditor employment, a reference for defended diplomas, a reference for developed study and lecture programs, a reference for teaching programs, a reference for participation in scientific research projects and relevant official notes are presented; reference for participation in international and national scientific forums, reference for additional requirements of Pl. University, order for participation in the accreditation committee at the PU, order for participation in a candidate student campaign, order for the quality committee at the Faculty of Bio-Logics at the PU, certificate for participation in the technical board of the j. Journal of BioScience and Biotechnology, diagnostics of protozoan parasites, disinfection, disinsection and deratization, certificate of completion of "Digital Skills" training and anti-plagiarism training in higher education institutions.

By order RD-22-440 of February 18, 2025. of the Rector of Plovdiv University (PU) "P. Hilendarski" I have been appointed as a member of the scientific jury of a competition for the academic position of 'associate professor' at the PU in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences (Zoology, Vertebrate Zoology), announced for the needs of the Department of Zoology, at the Faculty of Biology, PU "P. Hilendarski". Only one candidate submitted documents for participation in the announced competition, Chief assistant Dr. Vessela Ilieva Mitkovska, Department of Zoology, Faculty of Biology, University of Plovdiv "P. Hilendarski ".

The presented by Dr. Vessela Mitkovska documents are 15 listed in detail in her application for participation in the competition, all of which are in accordance with the Rules for the Development of the Academic Staff of the PU.

The candidate Ch. assistant Dr. Vessela Ilieva Mitkovska has contributed a total of 35 scientific works: Co-authored by: 1. Guide for exercises and tests on "Zoology of Vertebrate Animals /2014,2018,2022/", ed., PK "Zhanet 45", Plovdiv; 2. Textbook on "Genetic bases of behavior /2011/","P. Hilendarski" University Publishing House, 3. A complex view of the relationship "Heredity - Environment - Male Reproductive Health". Ed. Perspectives, Plovdiv and a list of 32 scientific research papers. 22 scientific papers are accepted for review, which are outside the dissertation and the obtaining of the academic position Ch. assistant. The distribution of scientific works by relevant headings, in the country and abroad, is as follows:1. V 4. Habilitation paper: Scientific papers in publications that are referenced and indexed in world-famous databases with scientific information (Web of Science and Scopus), a total of 6 issues;2. G 7. Scientific paper in publications that are referenced and indexed in world-renowned databases with scientific information (Web of Science and Scopus), a total of 15 issues. 3. A book chapter of a collective monograph was published, 1 issue.

All submitted documents are in accordance with article by low 4 (3) of LDAMRB article by low 2 (2) article by low 57 (2) of PRLDAMRB and art. 6 (2), PRDAM and all submitted documents are very well arranged and prepared.

1. Brief biographical data of the applicant

Ch. assistant Dr. V. Mitkovska was born in 1977. In 1994 graduated from secondary education in Plovdiv, and in 1999 received higher education in Pl. University, MSc, "Biology", specialization "Genetics and Microbiology". From 2003-2011 works as a biologist junior expert in the Department of "Zoology", Biol. Faculty of PU, prepares laboratory and field exercises in Vertebrate Zoology and Animal Diversity. Since 2011 – 2015 she is an assistant in the Department of Zoology, conducts laboratory and field exercises in the disciplines: "Vertebrate Zoology", "Animal Diversity" and "Medical Zoology". In 2014 successfully defended her Doctor Thesis in the field of zoology and received the educational and scientific degree "Doctor". Since 2015 until now she is the Ch. assistant at the Department of Zoology, Pl. University, "P. Hilendarski", conducts laboratory and field exercises as well as lectures in the disciplines: "Vertebrate Zoology", "Animal Diversity", "Medical Zoology" and has a supervision of graduates. In the "Zoology" department of the Faculty of Biology at PU, “P.

Hillendarski" she has a total of 23 years, 4 months and 27 days, of which 13 years 09 months and 27 days as a teacher at the same department and university.

2. General characteristics of the candidate's activities

Evaluation of the candidate's teaching and pedagogical activities and preparation (teaching materials, lecture courses, work with students, graduate students and doctoral students)

From 2000 to 2025, Dr. Mitkovska has a significant auditorium occupancy, and for the period 2020 to 2025, it is expressed in a total of 3651 hours: 756 hours of lectures; 101 hours of seminars; 2,794 hours of exercises / lectures are equated to exercises - 1 hour of lectures = 2 hours of exercises/.During the above five academic years, she has taught classes included in the curriculum of the following master's programs (MP): MP "Genetics" - practicum on "Methods for reporting cyto- and genotoxicity" with a teaching schedule of 20 hours; MP "Archaeology and archaeological cultural heritage" - lectures and exercises on the subject "Zoology in archaeological research" with a timetable of 10/10 hours, at the Faculty of Philosophy and History, PU "P. Hilendarski". She has developed 6 courses for the Bachelor's degree program / "Zoology of Vertebrates", "Medical Zoology - Part II", "Electronic lecture courses and e-courses for exercises in "Zoology of Vertebrates", "Computer literacy - office documents and software" for a total of 10 specialties. She has also developed three courses for the Master's degree program / "Methods for assessing cyto- and genotoxicity" / "Zoology in archaeological research", "Computer programs in parasitology" for a total of 3 specialties. Her auditorium occupancy by year is as follows: 2020-2021. total 851 hours (156 hours of lectures; 695 hours of exercises); 2021-2022. total 693 hours (140 hours of lectures; 30 hours of seminars; 523 hours of exercises); 2022 -2023. total 723 hours (140 hours of lectures; 56 hours of seminars; 527 hours of exercises); 2023-2024 - 636 hours (136 hours of lectures; 500 hours of exercises); 2024-2025 - 748 hours (184 hours of lectures; 15 hours of seminars; 549 hours of exercises). Under her leadership, a total of 7 graduates have successfully defended their theses, of which 6 in the Bachelor's Degree and 1 in the Master's Degree, with the graduates' theses being in the field of zoology. She is a co-author of a handbook to vertebrates, 2022, containing 13 main topics, which include a theoretical part, tasks and tests. Together with colleagues from the Faculty of Biology of the University of P U she has published a textbook on "Genetic Basis of Behavior, 2011". Also, she is co-author of a book "A complex view of the relationship "Heredity - Environment - Male Reproductive Health, 2021". Ed. Perspectives, Plovdiv. She participated as a field expert in a project funded by the OP "Environment 2007-

2013". She also participated in the implementation of tasks set out in the National Program for Prevention and Vector-Borne Transmissible Infections in Humans in the Republic of Bulgaria. Dr. Mitkovska has significant administrative and organizational activities: accreditation committees, committees on quality of education at the Faculty of Biology at the University of Plovdiv; Academic mentor of students on various projects; Participation in a total of 9 organizing committees for holding scientific conferences. Over the past 5 years she has been a course supervisor for students majoring in Biology; Member of the Academic Council of the "P. Hilendarski" University since 26.05.2023; Member of the Faculty Council of the Faculty of Biology from 2018 to the present; Secretary of the Department of Zoology from 2011 to the present. She successfully uses English, Russian and French languages.

3. Evaluation of the candidate's scientific and applied scientific activities

All presented publications, 22 in total, are in English, and are in foreign journals, such as Ecotoxicology and Environmental Safety - 1 issue, Environmental Science and Pollution Research - 2 issues, Journal of Medical Virology - 1 issue. Apidologie - 1 issue. Heliyon - 1 issue, Problems of Infectious and Parasitic Diseases - 2 issues. There are also publications in the journal Acta Zool. Bulgarica - 10 issues and Ecologica Bulgarica - 4 issues. The total impact factor of all journals where the candidate has published is 28.361 with a total SJR 8.557. Her works are co-authored, which is determined by their complex nature of research. She is the first author of 9 scientific papers, second and third author in 3 publications, and a follow-up author in the remaining papers. Dr. Mitkovska has also participated with 16 papers at international forums, presenting a total of 4 reports and 12 posters; she has also participated with 5 reports and 15 posters at national forums with international participation; her participation in national forums is also active - 7 reports and 5 posters. All her participations are in English. The report on compliance with the minimum national and additional faculty requirements shows that her results exceed the requirements, collecting 512 points. They are distributed by indicators as follows: indicator A - 50 points; indicator V - 120 points; indicator G - 242 points; indicator D - 100 points.

Contributions (scientific, applied science, applied) and citations

The candidate's scientific contributions can be presented in 3 separate areas: 1. Biomarkers for evaluation of the genotoxic and cytotoxic potential of the environment in different species of zoomonitors; 2. The role of vertebrates as vectors and reservoirs of viral zoonoses and blood parasites; 3. Other studies of species-specific characteristics in mammals.

I. Biomarkers for the evaluation of the genotoxic and cytotoxic potential of the environment in different species of zoomonitors

In this line of research, the candidate has made significant original scientific and applied contributions. She used two biomarker tests in combination: the micronucleus test, as an indicator of chromosomal instability, and the Comet assay, as a biomarker for chronic and irreversible damage and disruption of DNA integrity, a marker for the earliest genetic "response" of populations due to chronic pollution. It should be emphasized that for the first time in Bulgaria, a molecular-cytogenetic method "Comet analysis" for the assessment of DNA damage has been used for biomonitoring purposes. This method has been successfully applied to zoomonitor species of small rodents of the genus *Apodemus,* genus *Mus* (Rodentia, Muridae) and genus *Microtus* (Rodentia, Arvicolidae). It establishes low levels of genotoxicity in chronically polluted environments, thus providing an early, adequate and prognostic assessment of the genotoxic stress and mutagenic potential of the environment. The established bioaccumulation and distribution of xenobiotics in the tissues and organs of small mammals are similar to those in humans, which allows predicting the genotoxic risk of the population in the studied areas with varying degrees of anthropogenic pollution (Scientific papers B.4.1, B.4.6, D 7.4, D.7.10, D.8.1). Using the two biomarker methods, the candidate conducted for a first time in the science genotoxicity study of chronic industrial and agrochemical pollution of surface waters in natural populations of the large water frog (*Pelophylax ridibundus*). Multiple DNA damage and nuclear abnormalities have been identified. It has been proven that heavy metal pollution has a higher genotoxic potential than pesticide pollution /Scientific papers B4.2, D7.12/. The results obtained indicate a more specific tolerance of amphibians to genotoxins in the environment. For the first time in the science, DNA damage has been recorded in spermatozoa of the honey bee *Apis mellifera*. Significant levels of DNA damage have been found in spermatozoa of bees isolated from bee populations. High levels of colony losses were recorded, indicating that environmental genotoxic agents may be responsible for disruptions in sperm DNA integrity. The study is original for the science and has important practical significance for the conservation of *A. mellifera* bees in the world /Scientific paper D.7.1/. An original protocol for sperm comet analysis of bees has been adapted and published, with the method modified due to the much more compact packaging of DNA with protamines. Comet assay of mature germ cells assesses long-term damage that may affect a number of bee offspring due to lack of DNA repair in mature spermatozoa /Scientific paper D.7.1/. The genotoxic and cytotoxic effects of maximum permissible concentrations of priority pollutants

in surface waters (chlorpyrifos, Ni, Pb, Cd) on the blood cells of young individuals of the common carp (*Cyprinus carpio*) species have been proven when exposed ex situ /conservation of components of biological diversity outside their natural habitats/. Numerous original results have been obtained, such as nuclear anomalies and induced DNA damage. A very well-pronounced cyto- and genotoxic effect on carp blood cells has been demonstrated upon exposure to the lowest maximum permissible concentration of Cd (0.45 μg/L) /Scientific papers B.4.3, B4.5, D7.11/. All results could be used as a basis for regulatory institutions and legislative bodies to develop studies to refine the permissible concentrations of pesticides and heavy metals in water bodies. The findings can be applied in environmental risk assessment and biomonitoring programs.

II. The role of vertebrates as vectors and reservoirs of viral zoonoses and blood parasites

The significance of the fluorescent stining acridine orange in the diagnosis of hemoparasites of the genera *Trypanosoma, Bartonella, Babesia* and *Hepatozoon* has been confirmed. This fluorescent technique is applied for the first time in Bulgaria and provides accurate diagnosis even of small intraerythrocytic forms of blood parasites. It allows for unerring reading of micronuclei, as well as for the detection of blood parasites /Scientific papers B.4.6, D.7.5, D.7.13/. For the first time, the circulation of *Dobrava* hantavirus in Bulgaria in the species yellow-throated wood mouse (*Apodemus flavicollis*), field mouse (*Apodemus agrarius*) and common vole (*Microtus arvalis*), and of *Puumala orthohantavirus* in the species rusty wood vole (*Myodes glareolus*), has been confirmed by real-time PCR and serological studies of rodents. /Scientific papers B.4.4, D.7.3, D.7.14/.Cities towns, so-called "hot spots" with a high risk of hantavirus infection for the human population, have been identified and a number of factors influencing the association of *Dobrava* hantavirus infections in *A. flavicollis* and *A. agrarius* have been assessed. Applying statistical methods of analysis, the candidate establishes a dependence on four factors: species, age, sex and reproductive status. This analysis has important implications for the human population demography and determines the real risks for people /Scientific papers D.7.3, D.7.14/. Dr. Mitkovska's research on the detection of the blood parasite *Trypanosoma musculi* in the blood of the Eastern Mediterranean house mouse *Mus macedonicus* is original. The important role of Eastern Mediterranean house mice as a reservoir of trypanosomal infection is confirmed /Scientific paper D.7.5/. For the first time in Bulgaria, a hemoparasite of the genus *Hepatozoon* has been reported in the blood of the large water frog (*Pelophylax ridibundus*) /Scientific paper D.7.13/.

For the first time in the country, infections with *Bartonella* spp., *Trypanosoma musculi* and *Babesia microti*-like piroplasma have been identified in four species of rodents. A possible relationship between blood parasites and micronuclear frequencies in erythrocytes of wild rodent populations from anthropogenically affected and background areas in Bulgaria has been assessed /Scientific paper B.4.6/.

III. Other studies of species-specific characteristics in mammals

The descriptions of the karyotype of two rare species of the Bulgarian mammalian fauna – the rice field mouse *Micromys minutus* (Pallas, 1771) and the western broad-toothed mouse *Apodemus epimelas* (Nehring, 1901) are original. Their cytogenetic characteristics are presented in detail (diploid chromosome set, chromosome morphology), and for the rice field mouse the location and distribution of constitutive heterochromatin, the number and arrangement of nuclear organizers in its chromosomes have been determined. Original results were obtained for reproductive parameters in the conditions of sympatry of the most common mesopredators in the country: the golden jackal and the red fox /Scientific paper D.7.7/.

The candidate has significant scientific activity, having submitted a report on a total of 7 national projects, 1 international project and 2 national programs under which scientific research activity has been carried out.

The papers of Dr. V. Mitkovska find a wide response among the scientific community. There are 50 citations of her publications, mainly in journals with impact factor and Q1. This fact speaks of the significance of the journals and the author's research published in them.

3. Assessment of the candidate's personal contribution

The candidate works on an exceptionally important problem of our time, environmental pollution and its impact on biota. The scientific publications presented by Dr. V. Mitkovska are collective, which allows for a comprehensive examination of the problem. She is an established teacher and researcher, with a clearly outlined pedagogical profile in the field of Zoology /vertebrate zoology/, with numerous original scientific and applied results in this field.

4. Critical remarks and recommendations

I would like to recommend to Dr. V. Mitkovska to organize the creation of a working group studding the impact of various environmental pollutants on important groups of vertebrates.

5. Personal impressions

From the few personal contacts I have had with Dr. V. Mitkovska, I have been left with the impression that she is an organized, ethical, and very hardworking person.

CONCLUSION

Based on the analysis presented in the review, I believe that Dr. V. Mitkovska meets the requirements of a member of the low 24 on the Development of Academic Staff in the Republic of Bulgaria and the Regulations for its Implementation, as well as the Additional Requirements of the Faculty of Biology for the Development of Academic Staff at the University of Plovdiv. The candidate's papers have original scientific and applied contributions that have received international recognition as they have been appeared in journals published by international academic publishers.

Her theoretical papers have practical applicability, and some of them are directly oriented towards academic work. The achieved results in educational and research activities fully comply with the minimum national and additional requirements of the Faculty of Biology of the Plovdiv University "P. Hilendarski", adopted in connection with the Regulations of the University for the implementation of the LDAMRB. After reviewing the materials and scientific papers presented in the competition, analyzing their significance and the scientific, applied science and applied contributions contained in them, I find it reasonable to give my high positive assessment and recommend to the Scientific Jury to prepare a report-proposal to the Faculty Council of the Biological Sciences at Plovdiv University "P. Hilendarski" for the election of Chief Assistant Dr. Vessela Mitkovska at the academic position of "Associate Professor" at the P. University the "P. Hilendarski" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, professional area: ZOOLOGY - ZOOLOGY OF VERTEBRATES.

18. 03.2025 Reviewer:

Prof. Dr. D. Sc. Paraskeva Michailova