To the Chairman of the Scientific Jury, appointed by Order No. 29 of 4 April 2025 of the Director of IBER-BAS

### REVIEW

On the defence of a dissertation for the acquisition of the educational and scientific degree of 'Doctor', in the field of higher education 4: 'Natural Sciences, Mathematics and Informatics', scientific field 4.3 'Biological Sciences', scientific specialty 'Ecology and Conservation of Ecosystems', by Niya Lyubenova Toshkova, a full-time doctoral student in the Department of Ecosystem Research, Environmental Risk Assessment and Conservation Biology at IBER-BAS, on the topic: Features of the Wintering of Cave-Dwelling Bat Species in Bulgaria in the Context of Global Climate Change, under the supervision of Prof. Dr. Vasil Valkov Popov (IBER-BAS).

**Reviewer:** Prof. Stefan Vacev Panaiotov, DSc

## Scientific specialty: Microbiology

#### Institution: NCIPD, Sofia

I declare that I have no conflicts of interest within the meaning of Art. 4, para. 5 of the Law on the Development of Academic Staff in the Republic of Bulgaria. I have no joint publications on the topic of the dissertation with MSc Niya Toshkova. This review has been prepared in accordance with the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations of IBER-BAS for the implementation of the Law on the Development of Academic Staff. I did not use artificial intelligence software to evaluate the dissertation.

#### I. General characteristics of the dissertation work

The submitted documents comply with the instructions published in the Regulations for the Implementation of the Law on the Development of Academic Staff in the Republic of Bulgaria, as well as with IBER-BAS's internal regulations for the implementation of the aforementioned law. The dissertation is 139 pages long. It includes the following main sections: Introduction; Goal and Objectives; Materials and Methods; Results; Discussion of the results; Limitations of the study; Conclusions; and Cited literature. The section 'Literature review, materials and methods, results and discussion' is unified and includes articles published in peer-reviewed journals. The cited literature is attached to the articles. Cited literature is also separately included in the sections 'Introduction' and 'Conclusion'. The dissertation is written in a professional style. It is easy to read. There is no unnecessary terminology, complex sentences or unclear expressions.

## II. Literary awareness and theoretical preparation of the candidate

The work presented, the relevance of the set goals, and the methods used for planning, conducting, and describing the research provide a clear indication of the candidate's awareness and theoretical preparation on the topic of the dissertation. The goals of the planned and conducted research are theoretically very well justified based on the literary data.

Due to their physiological characteristics, bats are particularly sensitive to environmental changes. Understanding how bats react to climate change is essential for assessing their vulnerability and developing effective conservation strategies. The PhD student Niya Toshkova emphasises that research in this area is limited, but she is successfully working in this direction.

### III. Methodology of the research conducted

Arousals from torpor is a physiological challenge for bats. Their oxygen consumption increases significantly. This can lead to increased levels of oxidants, causing imbalance, oxidative stress, damage to biomolecules, cell death, and tissue damage. Studying oxidative stress markers is therefore key to understanding the physiological processes that occur during hibernation, as well as providing information about the general health of bat species. In response to this knowledge gap, the PhD student is focusing on the winter activity of bats in Bulgaria, thereby laying the foundations for systematic ecophysiological research in this area. The methodology for studying the physiology of bats when they are aroused from hibernation (torpor) is innovative and appropriate.

The research objectives are clearly defined. The PhD student has set three complex goals: (i) determining the winter activity of bat colonies and its relationship to temperature fluctuations in the external environment, (ii) determining the health status of bats before and after the hibernation period, and (iii) supplementing the methods for assessing the effects of climate change on bats. The tasks set out to achieve these goals are adequate.

To assess the condition of bat wing membranes visually, the PhD student proposes photographing bat wings and performing a detailed visual analysis of the photos using ImageJ software. This enables a careful, in-depth study of wing health, the collection of quantitative data on various anomalous structures, and the determination of the wing damage index (WDI).

## IV. Significance and conviction of the results, interpretations and conclusions obtained

Each of the articles attached to the dissertation is accompanied by a summary in Bulgarian language. The articles are also included in their original English versions. The statistical methods applied to analyse the data support the reliability of the results obtained. The visualisation of the data is professional. The diagrams attached to the articles aid the interpretation and understanding of the results and conclusions. Most of the data has been deposited in international, publicly accessible data repositories. The studies conducted on the acoustic monitoring of winter bat activity, the measurement of temperature fluctuations, and the direct counting of bat colonies are large-scale and innovative for Bulgaria. The results of monitoring the health status of the studied bat populations before and after hibernation, as well as the influence of activity on key physiological parameters [such as body mass index (BMI), wing damage index (WDI), fur condition index (FCI) and dental condition index (DCI), ectoparasite load by visual observation and histological methods, and molecular markers of oxidative stress (malondialdehyde (MDA), glutathione (GSH) and DNA damage (8-OhdG)] are studies with fundamental contributions.

Studies aiming to predict the consequences of climate change on European bat populations are few. The lack of data hinders the development of preventive strategies to mitigate possible negative impacts on bats. In addition to a national monitoring scheme for cave-dwelling bats, Niya Toshkova recommends introducing a methodology that will allow detailed, long-term data on climate change-related threats to be collected.

### **V.** Evaluation of scientific contributions

The generalized conclusions from the results obtained on the activity, nutritional spectrum and health status of bats in winter before and after hibernation have original scientific, societal, agricultural and economic value as a whole. The PhD candidate deposited empirical data in repositories and proposed a new methodology for collecting data to assess threats related to climate change and their impact on bat populations. I acknowledge the references used to support the scientific conclusions, most of which are original. I also accept the PhD candidate's critical remarks on the study's limitations.

# VI. Assessment of the quality of the scientific publications reflecting the dissertation research

All six of the published and presented scientific works fully correspond to the dissertation's topic, goals and objectives. The PhD candidate Niya Toshkova, has successfully fulfilled all the set goals and objectives. The results obtained make a significant scientific contribution to the dissertation topic.

### VII. Critical remarks on the dissertation

I have no significant remarks or recommendations for the PhD candidate. As a minor point, however, I would note the large number of goals and objectives of the dissertation. Niya Toshkova could include additional articles to support these. She has included the minimum number of articles required for the defence and has other valuable publications on the dissertation topic. She has underestimated her own contributions to the topic of the dissertation.

## **Conclusion:**

From the materials presented in the dissertation, it is evident that Niya Toshkova has demonstrated diligence and scientific dedication, and that the research is her own work. I strongly recommend that the Scientific Jury award Niya Lyubenova Toshkova the scientific and educational degree of "Doctor" in the field of higher education 4. 'Natural Sciences, Mathematics and Informatics', scientific field 4.3 'Biological Sciences' and the scientific specialty of 'Ecology and Ecosystem Protection'.

**Reviewer:** 

Prof. Stefan Panaiotov, DSc