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REVIEW

on Dissertation thesis for obtaining the educational and scientific degree "Doctor" in the professional field 4.3. "Biological sciences", scientific speciality: "Ecology and conservation of ecosystems"

Author of the thesis: **Antoniya Yuriyeva Hubancheva**, PhD student at the Department of Animal Diversity and Resources of the Institute of Biodiversity and Ecosystem Research/Bulgarian Academy of Sciences (IBER/BAS).

Scientific supervisors: Dr. Holger R. Goerlitz (Institute of Ornithology, Max Planck, Germany) and Prof. Dr. Dragan Petrov Chobanov (IBER/BAS)

Thesis topic:

"Sound Based Predator-Prey Interactions Between European Bats and Bush-Crickets"

Reviewer: Prof. Dr. Diana Peneva Zlatanova, PhD, Faculty of Biology/Sofia University "St. Kliment Ohridski"), member of the jury for awarding the educational and scientific degree "Doctor", according to the Order No. 76/06.10.2023 of the Director of IBER/BAS

Documents submitted for final defence

The set of materials and documents presented in digital format complies with the requirements of the BRAADB Act, the Regulations for its implementation and the Regulations for the conditions and procedure for the acquisition of scientific degrees and academic positions at the Institute of Biodiversity and Ecosystem Research at BAS. The set includes:

- Dissertation (in English);
- Thesis Summary (in English);
- Reference for the scientific contributions of the dissertation;
- List of publications on the topic of the dissertation;
- Copies of publications on the topic of the dissertation;
- List of participations in scientific forums with papers and posters reflecting the research of the dissertation;
- CV, including a full list of publications;
- Bachelor's and Master's degree diplomas, certified by the Scientific Secretary;
- Report on the study process and credits obtained, certified by the Scientific Secretary;
- Minutes of the meeting of the Scientific Council of IBEI-BAS, for referral to defence and selection of the scientific jury;
- Abstract of the thesis in English, containing the title of the thesis and the main points of the thesis, intended for publication on the IBEI-BAS website.

General presentation of the candidate

Antoniya Yuriyeva Hubancheva graduated from the Faculty of Biology of Sofia University "St. Kliment Ohridski" in 2010 with a Bachelor of Science in "Ecology and Environmental Protection". In 2012 she obtained her Master's degree with a thesis entitled: "Nutritional analysis of two sympatric bat species (Mammalia: Chiroptera) *Myotis myotis* (Borkhausen, 1797) and *Myotis blythii oxygnathus* (Monticelli, 1885)". In this regard, it should be noted that the title mentioned in the CV submitted in the documentation of the thesis defence does not correspond to the actual title of the defended Master's thesis mentioned above.

In the period 2017 – 2023, Antoniya Hubancheva was a full-time PhD student in Ecology and Ecosystem Conservation at the Department of Animal Diversity and Resources, Institute of Biodiversity and Ecosystem Research, BAS. During her PhD, she continued her research on the order Chiroptera (Bats). During the period 2008 to 2023, she gained impressive experience in a number of scientific, applied and conservation institutions such as: Bat expert at the Bulgarian Biodiversity Foundation (2008-2009); Bat expert at the project "Mapping and determination of conservation status of natural habitats and species in Natura 2000", Lot 5 (Bats) of the Ministry of Environment and Water (2011-2012); Intern at the Pacific Island Ecosystems Research Center, United States Geological Survey, Hawaii, USA, Habitat Selection in the Endangered Hawaiian Hoary Bat project (2012); Field assistant and later as researcher at the Research Center, Tabachka, at the Sensory Ecology Group of the Max Planck Institute for Ornithology, Germany (2008-2012).), field project "Comparative diet analysis of the lesser and the greater mouse-eared bats"; guest researcher at the University of Bern, Switzerland, project "Niche release in an allopatric population of the lesser mouse-eared bat in Crete" (2013.); intern at Smithsonian Tropical Research Institute, Panama, project "Unlocking the Mysteries of Sleep - Improved Learning as a Shared Functional Benefit" (2013); research assistant at University of Bristol, UK, project "African Bat Conservation based in Liwonde National Park, Malawi" (2014-2015); Associate researcher/expert on bats at the National Museum of Natural History, Bulgaria (2008 - 2017); PhD student/researcher at the Acoustic and Functional Ecology Group, Max Planck Institute for Ornithology, Germany (2017 - 2022); Research assistant at the Marine Bioacoustics Lab, Aarhus University, Denmark (2022 - 2023). During these 15 years, Antoniya Hubancheva managed to gain considerable experience from ecological research in different parts of the world and a variety of species, mainly focusing on the order Chiroptera. From 2009 to 2023, the PhD student has participated in 14, mostly international conferences. For her work, she has also been awarded several recognitions and awards, such as "Best Presentation award, 2nd place, Meeting of German Bat Researchers, Greifswald, Germany" (2023); "Best presentation award 1st World Bat Twitter Conference #WBTC1 (2020)", distinction as one of the top 10 young people of Bulgaria, in the category" Moral and Environmental Leadership" (2016), as well as several scholarships and grants for research trips and project participation.

So far, according to the submitted documentation, Antoniya Hubancheva has six published articles and one in review. All this shows that the PhD student is already a young but

promising and internationally visible scientist, who can conduct independent research based on acquired interdisciplinary methods, knowledge and accumulated own experience.

Comment: It is appropriate and customary for the candidate to submit a CV in Europass format. The current CV format does not allow for her complete presentation due to missing data (date of birth, high school education, experience and skills, language skills, etc.).

General characteristics of the dissertation - volume and structure.

The dissertation is presented in English because one of the supervisors is a foreign scientist.

The work contains a total of 80 pages and is presented in a well-established model applied at IBEI - BAS - a dissertation in the form of linked articles. It includes Introduction - 1 page; Table of Contents - 1 page; Introduction - 3 pages; Aim and Objectives - 1 page with one aim and three objectives; Literature Review, Materials and Methods, Results and Discussion - 56 pages with two publications (corresponding to the first and second objective) and one preprint (version of a scientific manuscript published on a public server before the formal peer review - corresponding to the third objective); Summary and main conclusions - 9 pages; Acknowledgements - 3 pages; Declaration of originality and reliability in Bulgarian and English - 2 pages;

For the Literature Review and Materials and Methods subchapters, the PhD student has indicated that these are included with the publications, which differs from normal IBEI practice. In such a case, **it is necessary to include all appendices of the publications that** comprehensively describe the Materials and Methods **with the dissertation**. The body text of the dissertation **also lacks the required scientific contributions** of the doctoral student in the developed work as they were included in the Thesis Summary. These are provided as a separate document.

The attached publications and manuscripts (given in the order outlined in the dissertation) include :

- Publication 1 - 2 figures, 2 supplementary materials provided, 50 references;
- Publication 2 - 6 video files, 3 figures with three additional figures to Figure 3, 1 supplementary material, 50 references;
- Preprint 1 - 2 figures, 59 references;

Relevance of the dissertation topic

The present dissertation examines a little-studied aspect of predator-prey relationships, namely the bidirectional acoustic sensory link between predators and their prey, such as bats and insects. In this regard, quite rightly, the PhD student initially focused on determining the feeding spectrum of two model species *Myotis myotis* and *Myotis blythii* (Publication 1) in Bulgaria using modern methods such as DNA metabarcoding, which currently provide the most accurate results for this purpose. Although the diet of *Myotis myotis* has been well studied by morphological methods across the distribution of the species, the published information is often only presented at the order level. This research contributes not only to comparison, but also to a refinement of information to family, and where possible to genus

and species level. On the other hand, the diet of *Myotis blythii* is much less well studied and this publication makes a major contribution.

The second step of the study looks at the movement and foraging behaviour of *Myotis myotis* in the wild, using miniature loggers (tracking devices). The hypothesis that the species makes immediate foraging decisions based on prey profitability and environmental changes is tested. These bats were found to have two strategies with similar catch rates for small airborne insects and large ground-dwelling insects but with much higher success rates for airborne insects (76%) than for ground-dwelling insects (30%). Despite the greater failure rate, ground-living insects provide much greater biomass as food than actively flying insects. Another important finding from the study is that bats use only one strategy per night, indicating that they adapt their foraging approach according to weather and ground conditions. It is concluded that despite higher risk, bats prefer hunting ground prey due to higher biomass gain per unit of hunting effort. At the same time, they may adapt their strategy switching from ground to aerial hunting when environmental changes reduce the profitability of ground prey.

The final step of the study focused on the behavioural response of prey species, such as the long-tailed locust *Tettigonia viridissima.* These locusts have different strategies for avoiding predators while courting mating partners, and these strategies change with the age and experience of the insects. Young males prefer predator avoidance to mating by hiding from predators acoustically for longer, whereas old males prioritize mating opportunities over predator avoidance. Thus, different ages approach risk differently to balance the costs and benefits of breeding and survival at different ages and thus ensure the species' well-being.

Knowledge of the published literature

Antoniya Hubancheva shows excellent knowledge of the existing published literature. Literature sources are well selected and discussed in the publications, responding accurately to the issues analysed or discussed. This demonstrates an excellent theoretical background, which is logical given the international experience gained working in various institutions.

Methodical approach

The three steps of the study successfully combine different disciplinary approaches - molecular techniques, methods of tracking by loggers and analysing the information, and behavioural experiments. One of the publications (Publication 2) uses a combined approach - tracking by loggers and molecular metabarcoding analysis. The methods used are well described and in detail, with a few exceptions noted below. These methodological approaches quite accurately meet the research objectives and lead to fundamental knowledge to improve understanding of the functioning of predator-prey relationships in a natural balance. At the same time, as part of the whole process, the hypotheses used are correctly formulated and correspond to the capabilities of the methodology used.

Significance and cogency of the results, interpretations and conclusions

The results obtained are very detailed and well-illustrated with figures. The discussion on them is logical and convincing, and the interpretations are well supported with examples from

published sources. Consistency in the steps of the study and their support with clear results give this study a sense of completion.

Summary and main conclusions

The findings and conclusions are correct and significant, but in my view have some technical weaknesses as outlined below.

Critical notes to the dissertation

The chapter Summary and main conclusions has some weaknesses that prevent it from fulfilling its necessary function, namely:

- The Summary is presented as an abstract of the publications with a repetition of the description of methods, quantitative data and the results from different parts of the publications. What is not achieved here is precisely what is most important - the extraction of the most important conclusions of each publication and, through discussion, the indication of how these conclusions meet the objectives. The text presented in the thesis: 1). does not sufficiently explain how the results achieve the stated objective and 2). largely leaves it to the reader to find the link between results and stated objectives. Instead, there is a repetition of texts from publications. At the same time, this is the place for drawing important conclusions to enable the link between the results in Publication 1, Publication 2 and Preprint 1. The most important function of this chapter is to answer how the results achieve the set objectives and hence achieve the set objective. This is largely not achieved, and I attribute this to a misunderstanding of the term in English (Summary = Abstract) and the meaning of this chapter in English (Summary = Summary with interpretation and making internal connections).
- Main Conclusions - the conclusions should be more concise (with less text) and each conclusion should be based on one important result. Currently, there are many discussion elements in the conclusions (except Conclusions 6 - 9) that make it difficult to read and to determine what is a conclusion and what is a discussion to it. The place for this discussion is in the preceding Summary chapter. For example, in Conclusion 2, the last sentence could be dropped, or its meaning could be included and synthesized in a smaller text, while much of the text is present in the Summary chapter.

Other technical notes

- The second article (Echolocating bats prefer a high risk-high gain foraging strategy to increase prey profitability):
  - In addition to the main publication, it is necessary to present in the dissertation all the supplementary parts, including Materials and Methods for each part of the study, as well as the supplementary figures presented in the Supplements, to get a complete picture of the methodological approach and the accuracy of the results. For this review, I had to search for additional information online. This information

may not be available at a later date to readers of the thesis in libraries and this makes this thesis incomplete despite the thorough work that has been carried out;

- In the Materials and Methods section of the publication published online, the description of the loggers lacks information on the Horizontal Dilution of Precision (HDOP) index values and threshold value used in the study. The HDOP determines the precision of the location of each location and track and is directly relevant to determining which habitat it is in - and hence, to establishing the effect of habitat on hunting strategies. When GPS tracking is performed, this information is critical to establishing positional accuracy and precision of the results obtained. Publications that include such tracking techniques must also include a range of HDOP values.
- Lack of Latin name of the test species at the beginning of the publication. It is only provided in the Results chapter, making it difficult to identify the target species from the outset.

- It is appropriate that the scientific contributions of the PhD candidate should appear in the main thesis as it was done for the Thesis Summary, rather than as a separate file, which makes the thesis incomplete when it is used in libraries.

Scientific contributions

The PhD student has submitted a total of seven contributions - six scientific/theoretical and one of scientific and applied character, which I accept in full. All the contributions are new, original and of important fundamental value. The scientific and applied contributions are essential to increase the resolution of bat diet studies based on molecular methods, the results of which are stored in a shared environment (open-access databases).

Scientific papers, reflecting the dissertation research

The dissertation is accompanied by two publications in English on the topic of the dissertation and one reprint, all published in co-authorship. In the first publication, published in Metabarcoding and Metagenomics (Q1, SjR 0.84, IF 3.24), Antoniya Hubancheva is the first author, in the second, published in eLife (Q1, SjR 4.25, IF 8.71) she is the second author. In the preprint, Antoniya is also the first author. All three papers are co-authored with an international team, and the PhD student has a leading role in these publications.

Thus, the recommended additional criteria for IBER/BAS degrees **have been exceeded**.

Personal participation

I have known Antoniya Hubancheva since her student years, when I had the opportunity to teach classes in which she participated. I observed her not only as a student but also during joint fieldwork on the same project in the Osogovo Mountains. Antoniya Hubancheva has always demonstrated exceptional diligence and ambition in working with vertebrates with a focus on bats. I think she is a great inspiration for other young scientists with her international experience as well as her extraordinary gift of communicating scientific problems and stimulating the interest of young colleagues to develop in science. Based on my impression and the materials provided for the final defence of the dissertation, I highly appreciate Antoniya's involvement in the published works, as well as confirming the quality of the

scientific product produced. I believe that the data collected, their processing and analysis are largely her merit, aided by the successful work of the international teams. This is confirmed unequivocally by the fact that Antoniya Hubancheva is the first or second author in the publications presented. Her involvement in these publications is documented in detail in each of them.

Thesis Summary

The Thesis Summary is provided in English and meets the dissertation content and requirements. It reflects the main points of the study by including the most informative figures from the publications and the preprint.

I have the following questions for the PhD student:

1. From the foraging analyses done by prey species and active choice analysis during the hunting of ground- and air-dwelling insects, how can you determine if your two subject bat species are opportunists or narrower specialists? What can you say about the breadth of their foraging niche?

2. Are there aspects of bat predation behaviour and anti-predation behaviour of prey that you have found to exist but have not included in this thesis due to a lack of sufficient data - and if so, what are they?

3. Do you intend to do any research on the effect of weather conditions on hunting success in bats and in which species? If so, which data sources do you plan to use?

4. What is the next research you will undertake with the knowledge and experience gained from the development of this thesis?

Reasoned conclusion

The dissertation of ANTONIYA YURIYEVA HUBANCHEVA is an important and contemporary interdisciplinary scientific research that contains scientific and applied results with original scientific contributions. Despite the remarks made, which are only technical, I believe that the PhD student shows excellent mastery of the chosen methods, is well acquainted with modern literature sources and can well interpret the obtained results. On this basis, I believe she has acquired the necessary practical and theoretical knowledge in her professional field.

Based on this conclusion, I propose to the Honourable Scientific Jury to award the degree of **Doctor of** Education and Science to ANTONIYA HUBANCHEVA in the professional field 4.3 "Biological Sciences" and the scientific speciality: "Ecology and Conservation of Ecosystems."

17.11.2023 г. Reviewer: ……

(Prof. Dr. Diana Zlatanova)