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O P I N I O N

From: **Assoc. Prof. Dr. Yordan Spasov Koshev,** Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences (IBER - BAS)
About: Evaluation of a dissertation for the acquisition of the educational and scientific degree "Doctor"; *Scientific field*: 4. Natural sciences, mathematics and informatics, *Professional field*: 4.3. Biological sciences; *Scientific discipline*: Ecology and ecosystem protection; Scientific institute: IBER - BAS; *Department:* "Ecosystem research, ecological risk and conservation biology"
PhD thesis: „ **Impact of pesticides on amphibian species from water bodies with varying degrees of anthropogenic influence in Central Bulgaria "**
PhD candidate: **Blagovesta Dimitrova Dimitrova**
Scientific supervisor: **Assoc. Prof. Dr. Simeon Lukanov**
The opinion was prepared in implementation of Order No. 22/07.03.2025 of the Director of IBER-BAS.

Amphibians are one of the most vulnerable animal groups worldwide. They have a unique life cycle that includes life in aquatic environments and on land. The permeability of their skin makes them particularly sensitive to environmental changes, including to the impact of various types of substances used in agriculture. Among the main threats to the group are habitat loss, pollution, climate change and disease. In the modern world, with the increasing continuous intensification of agriculture, there is an increasing need to study the impact of agricultural techniques and chemicals on animal populations. In Bulgaria, there is a lack of similar research on amphibians, which is why the current dissertation is relevant and of high importance.

The doctoral student skilfully handles literature. However, the literary sources are cited in different styles, sometimes with abbreviations of the journal titles (e.g. J Zool, Environ Sci Technol), elsewhere with full spelling. An important title is missing, that is mandatory for every Bulgarian student and doctoral student entering the problem - Georgiev D. et al. 2011. Water pollution and impact on ecosystems, University Publishing House "Paisii Hilendarski", 153 pages. ISBN 978-954-423-721-9.

The doctoral student has correctly formulated the goal and objectives and has prepared working hypotheses in accordance with the unsolved problems presented in the literature review. The methods used here are fully adequate and correlated with the results obtained.

The main contribution of this dissertation work is in its scientific-applied nature. For the first time in our country, such a detailed characterization of the impact of pesticides on frogs has been carried out. Scientific contribution No. 5 is controversial, because the impact of azoxystrobin, one of the main ingredients of Ortiva ® TOP SC, has already been studied on the tadpoles of the common frog (*Rana temporaria*) (Bundschuh et al. 2021).

The doctoral student has presented one publication in which he is the lead author and two in which he is the third author. Two of the publications are with quartile Q1, which indicates excellent quality of the results obtained. I believe that it is a matter of time before the journal *Ecological Frontiers* receives the due impact factor and quartile (Q1) of its predecessor *Acta Ecologica Sinica*.

The second presented publication "Popgeorgiev G., Lukanov S., Dimitrova B., Naumov B. 2024. Importance of agricultural areas for the distribution of protected amphibian species in Bulgaria. Acta Zoologica Bulgarica, 76 (1): 87-96" is based on data up to 2022. In total 4231 records from online database platforms (*SmartBirds* Pro and GBIF) and 14 unpublished records

were used. The three co-authors of the doctoral student are leading herpetologists in our country and have been working with the studied species for decades. The lead author (Prof. Dr. G. Popgeorgiev) is the inspirer, founder and chief administrator of the SmartBirds Pro database and application (4081 records), a herpetologist and one of the best Bulgarian experts in GIS processing and analysis of such type of data. In this regard, can the doctoral student explain in more detail her personal contribution to the mentioned publication?

Above I have noted some critical remarks that I hope to receive an answer to. Here I will mention a few more basic remarks and questions for the doctoral student:

1. Why were the same species of frogs found in the experimental and control sites not used in the laboratory experiments?
2. Why was the fungicide Ortiva® TOP SC chosen for the laboratory tests, since there is information that the preparation has a harmful effect on aquatic organisms? The safety data sheet states that the substances in the mixture have a short-term (acute) and long-term (chronic) hazard to the aquatic environment (Category 1), as the preparation is very toxic to aquatic organisms (H400) and very toxic to aquatic organisms, with long-term effects (H410). Thus, the results obtained are basically confirmatory in nature.
3. The introduction and discussion do not address the publication by Bundschuh et al. 2021. Bottom-up effects of fungicides on tadpoles of the European common frog (*Rana temporaria*). Ecology and Evolution, 11: 4353–4365. The authors used a mixture of fungicides, including *azoxystrobin* – the main active ingredient of Ortiva® TOP SC, demonstrating the negative effect of the preparation on the food environment of tadpoles of the European common frog (*R. temporaria*).

The doctoral student has fulfilled the minimum according to the national scientometric requirements, namely, under indicator "A", he has submitted a dissertation for the award of the educational and scientific degree "doctor" (50 points) and under indicator "D" - he has submitted three publications (47 points).

I know Blagovesta Dimitrova since she joined the IBER-BAS as a biologist and then as a full-time doctoral student. I have wonderful impressions of her theoretical preparation, field experience and self-discipline in the timely completion of the assigned tasks. Together we have carried out various field studies of mammals in Bulgaria. My personal impressions of her work are that she demonstrates independence, perseverance and purposefulness in scientific research. The notes and recommendations made are not intended to belittle the dissertation work, but to clarify the design of the experiment and the contribution and merits of the doctoral student herself.

The dissertation work of Blagovesta Dimitrova represents a modern scientific study with original scientific and applied scientific contributions. In terms of structure and content, it fully complies with the requirements of the Law on the State of the Republic of Bulgaria, the Regulations for its implementation and the Regulations on the conditions and procedure for acquiring scientific degrees and for occupying academic positions of the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences.

I positively evaluate the dissertation work of Blagovesta Dimitrova Dimitrova and I strongly support her awarding of the educational and scientific degree "Doctor" in the professional field 4.3. Biological Sciences (Ecology and Ecosystem Protection).

08.05.2025г. Opinion prepared by:
Sofia /Assoc. Prof. Dr. Yordan Koshev/