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STATEMENT

by Dr. Tsenka Georgieva Tchassovnikarova

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Regarding: PhD thesis for the award of the educational and scientific degree "**Doctor"** by the field of higher education **4. Natural Sciences, Mathematics and Informatics,** professional field **4.3. Biological Sciences,** announced for the needs of the Department of "Ecosystem Research, Ecological Risk and Conservation Biology," Section "Community Ecology and Conservation Biology", department "Ecosystem Research, Ecological Risk, and Conservation Biology" at the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences.

Thesis title: "**Pheromone communication, functional morphology of reproductive organs, and mating behavior in snakes of the family Viperidae in Bulgaria**"
Name of the doctoral candidate: **Kostadin Ivanov Andonov**
Scientific advisor: **Prof. Dr. Borislav Yasenov Naumov**

1. General description of the materials presented

The set of materials presented by K. Andonov complies with the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations for its application, as well as with the Regulations on the conditions and procedures for acquiring scientific degrees and occupying academic positions at the Institute of Biodiversity and Ecosystem Research (IBEI) at the Bulgarian Academy of Sciences (BAS). K. Andonov's dissertation is presented in the form of four bound scientific publications. He is the first author of all four articles, which are published in authoritative journals indexed in WoS and Scopus, as follows: Molecules - JCR-IF (2020): 4.412; Q1 (Scopus), Biochemical Systematics and Ecology - JCR-IF (2023): 1.4; Q3 (Scopus), Herpetozoa - JCR-IF (2024), Q2 (Scopus) and Acta Herpetologica, - JCR-IF (2024), Q2 (Scopus).

2. Relevance of the topic and significance of the research

Chemical communication and sexual behavior in snakes are areas that remain poorly studied, especially in European species. Despite significant progress in research on North American species, data on the specific chemical composition of skin secretions, the functional role of long-chain methyl ketones, and the relationship between chemical signals, mating behavior, and the morphology of the reproductive organs of European representatives of the genus Vipera are

practically absent from the scientific literature. The current trend toward integrative approaches combining biochemical, behavioral, and morphological methods emphasizes the importance of such studies for revealing evolutionary mechanisms in reproduction. Given the limited data on *Vipera ammodytes* and *V. berus*, as well as the lack of previous studies on snake chemical secretions in Bulgaria, the topic is highly relevant. It corresponds to priority areas in evolutionary biology, herpetology, and chemical ecology.

This study is of high scientific significance as it combines three key areas—chemical ecology, behavioral biology, and functional morphology—which are rarely considered in an integrated context for European snake species. The study provides the first data on the lipid and ketone composition of skin secretions in many species, including those for which no such information was previously available. The manifestation of specific behavioral responses to individual methyl ketones, as well as the detailed comparative morphological analysis of the reproductive organs, reveals new aspects of the reproductive biology of these species and contributes to the overall understanding of the evolutionary mechanisms that shape chemical communication and sexual selection in reptiles. In this sense, the work represents an essential step towards building a more complete picture of chemical signaling in European snakes.

3. Contributions of the dissertation

The dissertation contains significant contributions. The skin secretions of 12 snake species have been studied and described for the first time, which significantly expands the available database in herpetology and chemical ecology. The identification of key long-chain methyl ketones and squalene in the skin secretions of *V. ammodytes* and *V. berus*, along with evidence of their involvement in sexual communication, represents an original scientific contribution with the potential to be further developed in future research. The behavioral experiments conducted establish specific reactions of male individuals to certain compounds and provide initial indications of the potential composition of the female sex pheromone. An additional contribution is the development and application of a methodology for the three-dimensional examination of female genitalia using silicone casts, which enables comparison with hemipene morphology and opens the door to functional interpretations. This approach is new to herpetological research in Bulgaria and contributes to a better understanding of snake sexual morphology.

4. Recommendations

Chemical analysis reveals important long-chain ketones, but the study focuses almost entirely on the non-volatile fraction of skin secretions, potentially underestimating the role of volatile compounds, which are known to be actively involved in sexual communication in other snake species. The use of more sensitive methods—such as GC×GC-MS, solid-phase microextraction (SPME), or time-of-flight mass spectrometry (TOF-MS)—would allow the detection of even more substances at low concentrations. In behavioral experiments, response interpretation is mainly based on licking frequency and a limited set of synthetic substances, providing a valuable but still partial insight into pheromone behavior in natural conditions. In addition, the work focuses on a single species (*Vipera ammodytes*) in laboratory tests, with the results extrapolated to *V. berus* without direct behavioral observations of the latter species, which limits the strength of the generalizations. The morphological studies lack functional experimental verification of the correspondence between male and female genitalia, which would provide more definitive conclusions about their role in sexual selection. These limitations do not diminish the significance of the results, but they do indicate directions in which future studies could build upon and complement the current conclusions.

CONCLUSION

The dissertation contains scientific results that represent an original contribution to science and meet all the requirements of the ZRASRB, the Regulations for the Application of the ZRASRB, and the relevant Regulations of the IBEI, BAS. The dissertation shows that the doctoral student, Kostadin Andonov, has in-depth theoretical knowledge and professional skills in Ecology and Ecosystem Conservation, demonstrating the qualities and skills necessary for conducting independent scientific research. For the above reasons, I provide an upbeat assessment of the research presented in the dissertation, the abstract, the results, and the contributions. I propose that the distinguished scientific jury award the educational and scientific degree of "Doctor" to Kostadin Ivanov Andonov in the field of higher education: 4. Natural Sciences, Mathematics, and Informatics, professional field 4.3. Biological Sciences.

Sofia, December 11, 2025 Assoc. Prof. Dr. Ts. Chassovnikarova