

**REVIEW**

by

Ivelin Aldinov Mollov, PhD - associated professor,  
Plovdiv University “Paisiy Hilendarski”, Faculty of Biology,  
Department of “Ecology and Environmental Conservation”,  
member of the scientific committee for awarding the educational and scientific degree "doctor"  
(PhD), pursuant to Order No. 95/24.10.2025 issued by the Director of the Institute of  
Biodiversity and Ecosystem Research – BAS.

About a dissertation for awarding the educational and scientific degree “doctor” (PhD) by:  
**Field of higher education:** 4. Natural sciences, mathematics and informatics  
professional direction, 4.3. Biological Sciences, doctoral program “Ecology and Ecosystems  
Conservation”

**Author:** Kostadin Ivanov Andonov

**Title:** Pheromone communication, functional morphology of genitalia, and mating  
behavior in snakes of the family Viperidae in Bulgaria

**Scientific advisor:** prof. Borislav Yassenov Naumov

This review examines the dissertation thesis presented for acquisition of the educational and scientific degree “doctor” (PhD) in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences, doctoral program “Ecology and Ecosystems Conservation”. Kostadin Ivanov Andonov is a part-time PhD student in scientific specialty 4.3. Biological Sciences (“Ecology and Ecosystems Conservation”), to the “Ecosystem research, ecological risk and conservation biology” department at the the Institute of biodiversity and ecosystems research, Bulgarian Academy of Sciences. The thesis was developed as part of a part-time doctorate at the IBER - BAS. The PhD student's documents were prepared, deposited and checked according to the accepted requirements of the Scientific Council of IBER at the Bulgarian Academy of Sciences.

**Short biography of the candidate**

Kostadin Andonov is an environmental scientist with over ten years of professional experience. He holds a Master’s degree in Zoology from Sofia University „St. Kliment Ohridski” with part of his studies completed at the University of Antwerp. Between 2018 and 2024, he worked at the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences as an expert in biodiversity in protected areas and species monitoring. He has supervised and participated in numerous scientific and applied projects.

He has published many articles in international scientific journals, primarily in the fields of herpetology, chemical ecology, and species distribution. In addition to his academic work, Andonov plays a leading role at WWF Bulgaria, where he works on projects that promote innovation and youth engagement in environmental conservation.

He is the founder and director of Bulgaria's first green pre-accelerator, „Panda Labs“ and also coordinates a number of international projects.

Since 2023, he has been part of the international research and consulting company „Ecoris“ where he works on policy evaluation, strategic analyses, and data management.

### **General characteristics of the dissertation thesis**

The content, structure, and format of the dissertation comply with the established requirements and include all necessary sections. The dissertation is written on 117 pages and is structured as follows: “Abstract” (1 page), “Introduction” (1 page), “Literature Review” (12 pages), “Aim and Objectives” (1 page), “Material and Methods” (13 pages), “Results” (5 pages), “Article 1” (17 pages), “Article 2” (15 pages), “Article 3” (12 pages), “Article 4” (12 pages), “Discussion” (3 pages), “Summary of Results” (1 page), “Conclusions and Recommendations” (2 pages), “Acknowledgements” (1 page), “Declaration of Originality and Authenticity” (1 page), and “References” (17 pages). The dissertation is composed primarily of four bound articles. It includes one table and six figures in addition to those presented in the articles themselves. The list of references cited in the dissertation comprises 156 sources, all in Latin script. All literature sources cited in the published materials are included in the respective appended articles.

The thesis summary is made according to the generally accepted requirements, contains 38 pages, contains basic information from the research and follows the basic structure of the dissertation.

I have not detected plagiarism in the dissertation and the thesis summary presented to me for review.

### **Literature awareness and theoretical preparation of the candidate**

The doctoral candidate demonstrates an excellent understanding of the issues addressed in the dissertation. He emphasizes all major aspects of the conducted research. A substantial number of literature sources are cited in the dissertation - 156 references, and an additional 269 sources are cited in the bound articles. In my opinion, no significant works have been omitted. The doctoral candidate has skillfully used the accumulated knowledge on the topic, which has been successfully applied in the interpretation of the results. He demonstrates fluent command of the specialized terminology. Throughout the work, his strong practical training and professional approach to the studied subject matter are clearly evident.

### **Methodical approach**

The study was conducted using a modern, appropriate, and well-applied methodology that enables the achievement of the stated aim and the successful fulfillment of the objectives. A substantial amount of fieldwork was carried out, as a result of which material was collected from various locations and from a sufficient number of individuals, ensuring the representativeness of the sample and the reliability of the subsequent analyses.

The use of established protocols for the extraction of skin secretions and the application of gas chromatography with mass-spectrometry represent a standard and scientifically sound approach to the identification of chemical compounds. The behavioral experiments were conducted in accordance with well-established scientific practices and included appropriate controls to minimize confounding effects. As behavioral experiments

with animals are often unpredictable and it is difficult to replicate a specific methodology used for other species, the doctoral candidate's adaptation and further development of existing methods in order to achieve better interpretation of the results makes a particularly positive impression.

The morphological studies of the genitalia employ both classical and modern techniques, allowing for reliable comparative analyses. The doctoral candidate skillfully uses a combination of methods to reveal a more detailed picture of the three-dimensional correspondence between male and female genitalia (silicone casts of female genitalia and preparations of hemipenises).

The combination of biochemical, behavioral, and morphological approaches provides greater completeness and, to some extent, interdisciplinarity to the study. The statistical methods were selected in accordance with the nature of the data, with multivariate and mixed models applied correctly, demonstrating a high level of methodological competence on the part of the doctoral candidate.

### **Significance and importance of the results, interpretations and conclusions**

The dissertation is impressive in terms of the depth and scope of the scientific research. The doctoral candidate has focused on a topic that is generally poorly studied - the reproductive biology of European species of the family Viperidae. The focus of the dissertation progresses from chemical communication, through mating behavior, to the anatomy of the reproductive organs, which also represent the main stages of the conducted research.

The results are original and interesting. For the first time, the chemical profile of skin secretions is presented for 13 different snake species from Bulgaria and other countries, including key ketones with potential pheromonal functions. The robustness of the results is strengthened by the application of an integrated methodological approach, including field studies, GC-MS analyses, behavioral experiments, and statistical analyses. The interpretations are logical and consistent, built upon comparisons with existing literature and appropriately accounting for the limitations associated with the complex nature of pheromonal signals.

The conclusions fully correspond to the stated aim and objectives and to the topic of the dissertation. Particularly significant is the conclusion that the absence of clearly expressed sexual differences in the relative concentrations of key compounds suggests a more complex, multicomponent chemical communication system in snakes of the family Viperidae. The analysis of the influence of hibernation on the chemical profile of skin secretions represents an important contribution with ecological and physiological relevance. The behavioral studies are examined with careful attention to detail, the specificity of the experiments, and the variability of the results.

The conclusions regarding the role of certain ketones in communication between individuals are supported by appropriate statistical models and analyses. The analysis of the morphology of the reproductive organs in both sexes contributes to a better understanding of functional compatibility between the sexes and builds upon existing descriptive data. Particularly convincing is the comparative aspect, including representatives of different snake families, which allows for broader interpretations.

Overall, the dissertation clearly demonstrates both theoretical and applied contributions to herpetology and chemical ecology. The results, interpretations, and

conclusions can be assessed as significant, scientifically sound, and convincing, fully meeting the requirements for a doctoral dissertation for the PhD degree.

### **Critical notes to the dissertation thesis**

The reference list should be numbered, as this would significantly facilitate the work of the reviewers.

### **Scientific contributions**

The formulated scientific contributions are divided into three groups: composition of skin secretions; mating behavior; and morphology of the reproductive organs. All of them accurately and correctly reflect the doctoral candidate's main achievements.

#### *Composition of skin secretions*

For the first time, 88 chemical compounds involved in the skin secretions of 12 snake species have been identified and described, with additional data provided for *Vipera berus* supplementing the study of Van Moorlegheem et al. (2020).

Long-chain methyl ketones (C25–C33), squalene, and other key compounds have been identified for the first time in the skin secretions of *Vipera ammodytes*, which, according to the literature, are involved in chemical communication in some snake species. The presence of such substances has also been confirmed in *V. berus*. Analogous compounds have been identified in the skin secretions of *Natrix natrix*, *Dolichophis caspius*, *Platyceps najadum*, *Zamenis situla*, *Zamenis longissimus*, *Elaphe quatuorlineata*, *Coronella austriaca*, *Malpolon insignitus*, *Montivipera bornmuelleri* and *Daboia mauritanica*.

The involvement of the above-mentioned methyl ketones in chemical communication and in the female sex pheromone of *V. ammodytes* has been established. Similarities in the concentrations of certain ketones between male and female individuals of *V. ammodytes* and *V. berus* have also been identified, suggesting complex behavioral adaptations and/or the presence of other substances involved in the female sex pheromone.

The key role of hibernation in *V. ammodytes* in the formation of ketones and other key compounds has been demonstrated.

#### *Mating behavior*

It has been established that male individuals of *V. ammodytes* exhibit more active exploratory (roaming) behavior compared to females. It has also been shown that male nose-horned vipers are more attracted to long-chain methyl ketones than females. It has been demonstrated that, similarly to other snake species, in *V. ammodytes* and *V. berus* females release a sex pheromone that induces active orientation of males toward the source of the chemical signal without any additional (e.g., visual) stimulus.

#### *Morphology of the reproductive organs*

For the first time, the morphological characteristics of female genitalia have been described for seven snake species from three families based on three-dimensional silicone casts. These species are: *V. ammodytes*, *V. berus*, *Eryx jaculus*, *Dolichophis caspius*, *Zamenis longissimus*, *Elaphe quatuorlineata* and *Coronella austriaca*. For the first time, a three-dimensional comparison between male and female reproductive organs

of the listed species has been conducted, with a particular focus on *V. ammodytes* and *V. berus*.

I accept all contributions presented by the doctoral candidate and define them as original as well as confirmatory contributions.

### **Scientific publications reflecting the dissertation thesis**

The dissertation includes a total of 4 publications on the researched topic, which in practice represent the essence of the study. The works are co-authored, with the doctoral candidate being the leading author in all of them. It is important to note that research of this kind would be extremely difficult to carry out by a single person alone, so teamwork is something completely normal and frequently encountered in modern research. All articles are published in prestigious international peer-reviewed journals. All four articles have an impact factor: MDPI „Molecules“ – Q1; „Herpetozoa“ and „Acta Herpetologica“ – Q2; and „Biochemical Systematics and Ecology“ – Q3. All four publications are entirely on the topic and reliably present the obtained results. In addition, the doctoral student has participated in one international and one national scientific conference – the „21st European Congress of Herpetology“ in Belgrade, Serbia, and the „Fourth Interdisciplinary Doctoral Forum with International Participation“ held in Sandanski.

The fact that two of the listed articles (those in „Molecules“ and „Biochemical Systematics and Ecology“) have already been cited several times indicates that the published results are relevant and generate interest within the scientific community.

### **Conclusion**

I do not personally know the doctoral candidate Kostadin Ivanov Andonov, so I assess his qualities as a scientific specialist and herpetologist solely based on the dissertation submitted for my review and the scientific articles published to date. In this regard, I can say that Kostadin Ivanov Andonov has established and proven himself as a very good specialist, meticulous researcher and analyst, exceptionally well-prepared herpetologist and ecologist, whose name is already well recognized by the scientific community.

During the dissertation period, the doctoral candidate has carried out a vast amount of field and laboratory work, as well as analysis and processing of the obtained results. A large amount of material has been collected based on modern, up-to-date methods. Valuable original results have been obtained, on the basis of which a number of contributions of an original and confirmatory nature have been made.

The dissertation demonstrates that the doctoral student Kostadin Ivanov Andonov possesses in-depth theoretical knowledge and professional skills necessary for obtaining the scientific and educational degree „Doctor“ (PhD) in professional field 4.3. Biological Sciences, in the scientific specialty „Ecology and Ecosystems Conservation“ as he demonstrates the qualities and skills for independently conducting scientific research.

Due to the above, I confidently give my **positive evaluation** of the research conducted, presented in the dissertation, abstract, achieved results, and contributions. Therefore, I propose that the members of the esteemed scientific jury vote "YES" the awarding of the scientific and educational degree „Doctor“ (PhD) in professional field 4.3.

Biological Sciences, in the scientific specialty „Ecology and Ecosystems Conservation“ to Kostadin Ivanov Andonov.

Plovdiv, 18.12.2025.

Reviewer:  
(Assoc. Prof. Ivelin Mollov, PhD)