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OPINION

on the PhD thesis of doctoral student Kostadin Andonov on the topic "Pheromone communication, functional morphology of the genital organs and mating behaviour in snakes of the Viperidae family in Bulgaria" for the acquisition of the scientific and educational degree "Doctor", Doctoral Program "Ecology and Conservation of Ecosystems"

Scientific supervisor: Professor Borislav Naumov

Committee Member: Professor Boyko B. Georgiev, PhD, DSc (IBER-BAS)

The dissertation by Kostadin Andonov is dedicated to the study of important and poorly studied aspects of the reproductive biology of two species of snakes of the family Viperidae, i.e. the horned viper (*Vipera ammodytes*) and the adder (*Vipera berus*). These are the only representatives of the family Viperidae for which there is currently reliable data that they occur in Bulgaria. The research approach is multidisciplinary – it includes approaches from ethology (characteristics of mating behaviour), comparative morphology (characteristics of female copulatory organs) and chemical analysis of skin secretions, which are considered to play an important role in communication between the sexes. In this way, poorly studied aspects of the reproductive biology of both species are covered. This is a modern direction of research, which, in addition to its scientific importance, is also of essential importance as a basis for understanding their biology and for building a conservation strategy for them.

The selected research methods are appropriate to the tasks set.

The results of the research are presented in the form of four articles, which were published in the renovated international journals *Herpetozoa*, *Acta Herpetologica*, *Biochemical Systematics and Ecology* and *Molecules*. The doctoral student is the lead author of these publications, which demonstrates his leading role in the research.

I would point out the more important, in my opinion, contributions of his studies:

- The substances involved in the skin secretion complexes of both species, as well as in 11 other species of snakes, have been identified.

- Long-chain methyl ketones (C25–C33), squalene and other key compounds have been identified in the skin secretions of both species, which, according to data from other authors, are involved in chemical communication in snakes.

- The participation of the identified ketones in chemical communication and in the female sex pheromone of *V. ammodytes* has been established.

- In *V. ammodytes*, differences in the concentrations of a number of compounds between active individuals and individuals that have fallen into hibernation have been established.

- It has been found that males of *V. ammodytes* are more attracted to long-chain methyl ketones than females.

- The female genitalia of the two studied species, as well as in five other snake species from the fauna of Bulgaria, have been described in a comparative aspect.

When reading the dissertation, one is struck by the logical presentation, the clear formulation of the objectives, the description of the methodological approaches, the professional handling of terminology and the reasoned conclusions. The illustrative material is carefully prepared and convincingly supports the results and conclusions of the research conducted.

The research presented in Kostadin Andonov's dissertation represents a significant contribution to the field of reproductive biology and ecology of snakes. On the other hand, these studies are also a significant contribution to the development of ecological research in the field of herpetology at the Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences. I believe that they are an important step in the development of a young and active researcher who demonstrates the necessary aptitudes for a fruitful scientific career.

Based on the above, I give a positive assessment of the dissertation research and support the awarding of the scientific and educational degree "Doctor" to Kostadin Andonov, while at the same time congratulating both him and his scientific supervisor, Professor Borislav Naumov, for the valuable research conducted and the scientific contributions achieved.

18 December 2025 Signature

Boyko B. Georgiev
Professor, PhD, DSc