STATEMENT

by **Assoc. Prof. Irina Schneider, PhD** from Department “General and Applied Hydrobiology”, Faculty of Biology to Sofia University “St. Kliment Ohridski”

About: Dissertation on the subject “Study of the spawning and feeding habitats of the sturgeons in the Bulgarian section of the Danube River” of PhD student Borislava Kostadinova Margaritova to Department "General and Applied Hydrobiology", Faculty of Biology to Sofia University "St. Kliment Ohridski" in professional field 4.3. Biological sciences, scientific specialty „Hydrobiology” (Ichthyology and Aquaculture)

Borislava Margaritova was enrolled as a full-time PhD student in the Department by Order №RD 20-316/05.02.2019, and by Order №RD20-440/17.02.2022 she has the right of defense after successfully passing the exams (Certificate № 79/07.08.2022 by the Dean of the Faculty of Biology to Sofia University "St. Kliment Ohridski") and implementation of all planned activities according to the individual plan, including conducting practical exercises in the discipline “Hydrobiology” covering the requirement of 45 study hours. She presented the results of her dissertation in four articles, three of which are in refereed journals with an impact factor and quartiles Q2, Q3 and Q4. PhD student Margaritova passed a procedure for preliminary defense of the dissertation on 21.07.2022 according to Order №RD38-387/13.07.2022 of the Rector of Sofia University "St. Kliment Ohridski" with a positive assessment and referral to official defense. Miss Margaritova has presented the results of her dissertation at 3 international and 3 national scientific conferences. PhD student Borislava Margaritova **meets the minimum national requirements for awarding the ESD "Doctor" in professional field 4.3. Biological Sciences, scientific specialty "Hydrobiology" (Ichthyology and Aquaculture)**. According to Indicators of group A (dissertation for the award of the ESD "Doctor") she submitted 50 points, and according to Indicators of group D (scientific publications in editions that are refereed and indexed in world-famous databases with scientific information) she submitted 47 points out of the required 30 points.

The subject of **the dissertation concerns extremely topical issues** related to **the current status of sturgeon fish populations** in the Bulgarian section of the Danube River - **one of the species included in the IUCN Red List and in the Red Book of Bulgaria** and one of the most ancient representatives of the ichthyofauna. Despite fishing bans and introduced stocking procedures, their populations have not yet recovered after overfishing in the 1950s; the hydrotechnical facilities built along the river; water pollution; the disturbance of habitats as a result of dredging. The long-life cycle of sturgeon fish, their late sexual maturation, the specific requirements for breeding grounds are some of the reasons for **the slow recovery of populations**. All these reasons require **a complete, complex and in-depth study** on the food spectrum of sturgeon fish, the characterization of feeding and breeding habitats, a study of migration routes, the introduction of measures for conservation and restoration of populations and conduct explanatory campaigns for the public and include part of the target groups in the problem solving. Such is the investigation, presented by the team of PhD student Margaritova and her scientific supervisors, Assoc. Prof. Eliza Uzunova, PhD and Assoc. Prof. Lyubomir Kenderov, PhD.

The dissertation of Borislava Margaritova, **in terms of volume, purpose and tasks, results, discussion and conclusions, fully meets the legal requirements** for this kind of scientific works. It contains the necessary chapters and has a total volume of 221 pages, of which 63 pages are presented in the form of an appendix, including additional visualization and proprietary data, and 2 pages of acknowledgments. The work is structured in the following sections: introduction /2 p./, literature review /31 p./, aim and tasks /1 p./, materials and methods /24 p./, results /36 p./, discussion /35 p./, conclusions /2 p./, contributions /2 p./, references /23 p./, consisting of 279 literary sources. The dissertation is richly illustrated through 19 figures, 21 tables, as well as with the figures and tables separated in an appendix. The dissertation contains scientific and applied scientific results, formed in 10 contributions of an original and confirmatory nature.

The introduction and literature review are purposeful, presented clearly and comprehensibly. The origin, distribution, biological characteristics and representatives of the order *Acipenseriformes*, as well as the state of the sturgeon populations in the Danube River, were studied. The applied legislative measures for the protection of populations and the measures for their recovery are analyzed. The lack of up-to-date data on: sturgeon fish, breeding ground and the food spectrum of sturgeons have been identified as **critical problems**. The purpose and tasks of the dissertation logically follow from the literature review. It is impressive that a large volume of data is included over **an 8-year sampling period**, which greatly exceeds the requirements for this type of work. The catch periods and sampling points, the number of samplings for the characterization of potential breeding habitats (2013-2019); the studies on sturgeon migration (2014-2021) and on the food spectrum (2019-2021) are presented in details. The monitoring is based on **a complex of physico-chemical indicators, quantitative and qualitative composition of the ichthyofauna and macrozoobenthos, size structure and length-weight dependences of sturgeons, the food spectrum of sturgeon fish, hydrographic survey of the habitats with validation of the sonar data by studying the bottom substrate**. Sturgeon migration and habitat determination were studied by **tagging fish** and analyzing data for catch frequency, length of stay in the study river section, growth rate, survival, and downstream movement speed. The nutritional spectrum was established by a non-invasive method through gastric lavage. Samples for macrozoobenthos were collected and processed, **952 specimens of sturgeon fish were examined** to determine species composition, abundance, size. Stomach contents were examined on a total of 78 specimens. Data processing includes **statistical methods**, and the result is presented clearly and comprehensibly in the form of figures and tables, and **the maps of the studied potential habitats** are made using ArcGIS 10.4.1.

The largest part of the dissertation is devoted logically on the results and discussion chapters, and on the related with them conclusions and contributions. Analyzing the data, PhD student Margaritova compares them with studies by other authors. The results and discussion chapters fully meet the purpose and tasks of dissertation, and include a study of: species composition, abundance, size and catch periods of sturgeon fish; potential sturgeon breeding habitats; sturgeon diet and feeding habitats. As a result of the analysis of the species composition, the dominant presence of *A. ruthenus*, followed by *A. stellatus* and single specimens of *H. huso* and *A. gueldenstaedtii* was established. The presence of hybrid species has been established as a result of share the same spawning grounds and have similar breeding behavior of two or more species, or as a result of sturgeon stocking. **A predictive model was applied to determine sturgeon hatching locations and timing**, which is based on temperature, water level, growth rate, and downstream movement speed of larvae and juveniles. A change in the composition of the food of young sturgeons was found, and instead of insects from the Trichoptera and Ephemeroptera, the amphipod *O. obesus* and the mussel *C. fluminea*, as well as *Chelicorophium spp.* and chironomids were ascertained. In the course of the discussion, the PhD student's style of **critical thinking and detailed discussion** of the obtained results is

demonstrated. The conclusions essentially reflect the results obtained. A strong impression is made of the **contribution nature of the present work**, on the one hand, to the **overall approach to sturgeon research**, and, on the other hand, to **the connection of the obtained results with the necessary measures to restore the sturgeon populations and the recommendations given to the state regulatory authorities**. The dissertation is an example of how the solving of complex environmental problems requires complex research approach and solutions. The synopsis accurately reflects and emphasizes the main points of the dissertation.

I have no specific remarks about the dissertation and about the research work of Miss Margaritova. I **recommend** of the PhD student, together with her supervisors, **to prepare subsequent review papers with the data presented in the dissertation**.

The dissertation shows that PhD student Margaritova has **theoretical knowledges and practical skills in the field of scientific specialty "Hydrobiology"** (Ichthyology and Aquaculture). The experimental design, the applied methods, the discussion of the results show that Miss Margaritova is a developed scientist, ready for independent scientific research, with acquired professional experience as an expert on water protection and species conservation in WWF Bulgaria. She has also participated in 13 research projects, some of them financed under the OP "Environment", LIFE, Transnational Cooperation Program "Danube".

Conclusion:

Based on the above, I consider that the required **educational and scientific criteria for awarding the ESD "Doctor" are fully covered**. Doctoral student Borislava Margaritova fulfilled her individual plan, and she is: mastered various hydrobiological methods, applied statistical methods for analysis and prognostic models, co-author of 4 scientific papers on the topic of the dissertation. **PhD student Margaritova is a researcher with clearly defined scientific interests who works well in a team**. The content of the presented dissertation on the subject: “Study of the spawning and feeding habitats of the sturgeons in the Bulgarian section of the Danube River” complies to the requirements of the Law for the development of the academic stuff in the Republic of Bulgaria and the Regulation for its application as well as the Regulation for the development of the academic stuff of Sofia University “St. Kliment Ohridski”. All of that gives me the reason to recommend with conviction to the honored Scientific jury as well as to the Scientific council of the Faculty of Biology to Sofia University “St. Kliment Ohridski” to **awarded to Borislava Margaritova the educational and scientific degree "Doctor" in the scientific specialty "Hydrobiology" (Ichthyology and Aquaculture) in professional field 4.3. Biological Sciences**.

08 December, 2022 Assoc. Prof. Irina Schneider, PhD