#### Вх.№ 523/НО-05/23.05.24 г.

#### **REVIEW**

# of a doctoral thesis for awarding the educational and scientific degree "doctor"

#### (PhD)

in professional field: 4.3. "Biological Sciences", scientific specialty: 01.06.11 "Hydrobiology"

Author of the PhD thesis: **Tsvetelina Yasenova Isheva**, regular PhD student at the Department of Aquatic Ecosystems of the Institute of Biodiversity and Ecosystem Research (IBER) at the Bulgarian Academy of Sciences (BASc) Scientific supervisor: Prof. Yordan Ivanov Uzunov, PhD (IBER-BASc, currently retired) Scientific consultant: Assist. Prof. Plamen Nikolaev Ivanov, PhD (IBER-BASc)

Title of the PhD thesis: " Study on benthic diatom assemblages in intermittent rivers in Southern Bulgaria and their application for assessment of ecological status"

**Reviewer:** Assoc. Prof. Ralitsa Petrova Zidarova, PhD, Department of Marine Biology and Ecology, Prof. Fridtjof Nansen Institute of Oceonology at the BASc, member of the Scientific Jury for awarding the educational and scientific degree "doctor" (PhD) according to Order No. 26/05.03.2024 of the Director of IBER-BASc

# Documents presented for final defense

The set of materials and documents (presented in digital format) complies with the requirements of the current Act on the development of the academic staff in the Republic of Bulgaria, the Regulations for its application and the Regulations for the terms and conditions for acquiring scientific degrees and for holding academic positions at the Institute of Biodiversity and Ecosystem Research at the BASc. The set consists of:

- 1. Application for admission to defense with list of documents
- 2. PhD thesis
- 3. Summary of the PhD thesis
- 4. Reference for the scientific contributions of the PhD thesis

5. List of publications related to the PhD thesis, citations and presentations at scientific forums

6. Scientific CV

7. Diploma for obtained master's degree (copy certified by the Scientific Secretary of IBER-BASc)

8. Protocol of the Meeting of the Department of Aquatic Ecosystems at IBER-BASc, directing the PhD thesis for defense with a proposal for a Scientific Jury

- 9. Reference for the study process and credits, certified by the Scientific Secretary
- 10. Abstract of the PhD thesis in English
- 11. Copies of scientific articles on the topic of the PhD thesis
- A full report of plagiarism check of the thesis was also provided.

# General presentation of the candidate

Tsvetelina Yasenova Isheva graduated with excellence from the Faculty of Biology of the St. Kliment Ohridski University of Sofia in 2011, with professional qualification Biologist-Master in Algology. Subsequently, she specialized in algal cultivation at Ghent University, Belgium, and freshwater algal identification at Durham University, UK. Currently, she is a biologist at the Department of Aquatic Ecosystems of IBER-BASc.

Between 2011–2023 Tsvetelina Isheva participated in a total of 14 mainly scientific and applied projects with national and external funding, which included hydrobiological studies and monitoring, development and updating of Management plans of natural and national parks in Bulgaria, intercalibration of methods for analysis of biological quality elements (BQE) for surface water types on the territory of Bulgaria, validation of the typology and classification system in Bulgaria for assessing the ecological status of surface water bodies from the "river", "lake" and "transitional waters" categories, and others.

During the time of preparation of her PhD thesis, according to the reference list submitted by herself, Tsvetelina Isheva has seven publications in scientific journals. Two of them are in co-authorship with renowned scientists from abroad, and in Q1 journals with a high impact factor in the year of publication, such as Science of Total Environment (IF2022/2023 9.8) and Protist (IF2012 4.063). It is obvious that Tsvetelina Isheva has gained significant experience in her work.

It should be mentioned that the presented Scientific CV by Tsvetelina Isheva does not follow the European format.

## General characteristics of the PhD thesis - volume and structure

The PhD Thesis of Tsvetelina Isheva is written on 194 standard pages, of them 41 pages of Appendices. It includes title pages (2 pages), table of contents (2 p.), list of figures and tables

(2 p.), abbreviations used in the work (1 p.), explanation of terminology (2 p.), projects related to the thesis (1 p., *note*: it is not indicated that these are projects, but it is obvious from the text), and the following chapters: Introduction (2 p.), Aim and Objectives (1 p.), Literature review (24 p.), Typology and characteristics of the studied rivers (17 p.), Materials (3 p.), Methods (13 p.), Results and Discussion (46 p.), Conclusions (4 p.), Contributions of the thesis (1 p.), Acknowledgments (1 p.), and References (30 p.). Thirty-one figures and 19 tables are nested within the text. The Appendices, after a blank title page (p. 153), include 11 plates with light microphotographs of the materials, as well as other six tables and one figure. The structure of the doctoral thesis is standard, and the studied area is presented separately as "Typology and characteristics of the studied rivers", which logically follows from the specifics of the work.

## **Relevance of the topic**

The topic of the doctoral thesis is among the always relevant topics, having both scientific and scientific-applied characters. Diatoms (Bacillariophyta) are one of the main biological elements used in the water quality assessment of surface waters (according to the Water Framework Directive, but also according to Bulgarian legislation, Ordinance No. H-4/14.09.2012). Intermittent rivers represent a significant part of the river ecosystems of the world; they are widespread in the Mediterranean region, and they provide very specific but also heterogeneous habitats. Globally, diatoms from this type of rivers are less studied, as well as their application for water quality assessment. The PhD thesis aims to study the taxonomic diversity, distribution, dominant structure, seasonal dynamics and ecology of diatom communities from intermittent rivers in southern Bulgaria, and to assess their ecological status, with the hypothesis that the results of the study will also confirm the need of update of the national classification methodology and scale for assessing the ecological conditions according to the "phytobenthos" parameter.

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

The list of references includes a total of 310 titles, most of them scientific publications in English, 11 normative documents, and 6 electronic sources. Tsvetelina Isheva shows a very good theoretical awareness and knowledge regarding the object of her study (diatoms), the scientific research carried out on diatoms from intermittent rivers around the world, the research on the river diatom flora in Bulgaria, as well as of the existing regulations.

#### Methodological approach

The methodology used by Tsvetelina Isheva for sample collection, sample laboratory treatment, qualitative and quantitative analyses of the diatom samples, and the interpretation of the samples, follows established European standards: EN ISO 13946:2014 (EN 2014a) and EN ISO 14407:2014 (EN 2014b), which are also adopted in our national legislation (Regulation No. 1/11.04.2011) for water monitoring. From the studied intermittent rivers, a representative number of samples (89) were collected from a large number of sites (49) during two hydrological seasons to ensure both statistical applicability and reliability of the data. The methodology is adequate for the objectives and nature of the PhD thesis.

# Significance and credibility of the obtained results, interpretations and conclusions

The study of the PhD student Tsvetelina Isheva is the first study of the benthic diatom flora of rivers of the national type R14 with an assessment of their ecological status during two hydrological periods. It provides new data on the diatom flora of this river type, but also on the Bulgarian flora as a whole. As a result of this research, the typology and the methodology for diatom samples collection and the classification system used for the assessment of ecological status based on the "phytobenthos" quality parameter have been developed, updated and intercalibrated, and adopted in both the National and European legislation. The results of the analyzes confirmed the hypothesis that the diatom communities of the studied river type differ significantly during the different hydrological phases and include indicator species that characterize individual classes of ecological status. As a result, it has also been confirmed that an update of the existing Regulation H-4/14.09.2012 is necessary. The research results are described in detail, and are well illustrated with figures and tables. They are widely discussed and interpreted using the currently available data from relevant literature. The following parts make a particularly good impression: Part 7.4. Development of a classification system for assessment of the conditions, 7.5. Assessment of the ecological condition of the studied rivers, 7.6. Diatom communities in excellent, good and moderate ecological condition. The results and steps leading to the update of Regulation H-4/14.09.2012 are very well described and presented. The conclusions of the work are logical. The objectives of the PhD have been achieved.

#### Critical notes to the PhD thesis

#References list should follow established standards, in order to be easier to find the source in the list. Usually sources in Cyrillic, even if few, are given first, followed by the cited sources in Latin, and without separating normative documents and electronic sources in other sections. When citing electronic sources, the most recent date on which they were accessed is usually indicated.

#In regard to Contribution 2: ....Detailed descriptions of the morphology, autecology and distribution of some of them have been made and they are documented with light and scanning electron microscope photographs. In my opinion, the contribution is in the provision of new and complementary information on a number of rare species, and not that description of these already known taxa was made, or simply in documenting them. That is, the contribution is there, but linguistically it could be better presented.

#Several technical errors have also been noted, for instance on p. 84, "presented in the exposition," which probably meant "presented in the Appendix"; also in Contributions, when citing the year of Regulation H-4 (but correctly cited in the Bibliography), etc. It is clear that avoiding all technical errors is difficult when dealing with large texts.

#As a tip for future work: split the results of the discussion, especially in scientific publications. Splitting results from discussion facilitates the understanding of the results, as well as their discussion.

Questions:

1. In the discussion on the finding of *Sellaphora madida*, the means of distribution and dispersal of diatoms are considered. Could the record of *Sellaphora madida* in Bulgaria be explained by "transportation" outside its natural range by animals, e.g. birds, or through human activity, e.g. aquaculture", or "with global warming, in which the living conditions of many organisms in the temperate climate zone become close to those of its native habitat", given that the other reports of this species come from very distant areas (USA and Brazil)? Does the finding of this species not actually speak of the insufficient studies of the habitats of the species, both in Europe and outside Europe? Is there a possibility that these are different lineages (American, European)? Are there any morphological differences between the American and Bulgarian populations?

2. In Conclusion 15: The low-profile and motile functional groups show a significant ability to discriminate between the ecological condition classes (pressure strength and direction, respectively), which proves that the majority of low-profile diatoms are indicative of nutrient-poor rivers in either excellent or good ecological condition, whereas most motile diatoms are indicative of increased nutrient levels, and river in a lower ecological condition. To what

extent could this distribution also be related to the degree of resistance of the two groups to water turbulence, i.e. current, grazing and potential grazers? Do the motile forms in the studied rivers also include small-celled motile species, or are they larger representatives of the group?

3. Page 21, "With regard to water resources, Bulgaria is ranked at one of the last places in Europe". It should be better explained. What exactly does this mean and what are the potential threats for our country?

4. It is interesting that at present the temporarily drying rivers provide rare and even threatened habitats, but on the other hand, many scientists predict that due to the climate changes, the number of these rivers may increase in future. Could we expect that in future the habitats provided by this type of rivers will no longer be rare, as well as the species inhabiting these habitats, respectively?

#### Nature of scientific contributions

A total of five scientific contributions are presented by Tsvetelina Isheva, which I accept, with the small linguistic note given above. Four of them are original contributions, of which two are floristic (i.e. scientific) and two in applied science, whereas the fifth contribution confirms previously published data. Contribution 3 has a very high value. It shows that Tsvetelina Isheva is an already established expert in hydrobiology, and the results of her work have led to changes and updates of existing legislations (Contribution 3: "The R14 national river type is aligned and intercalibrated with European types R-M1 and R-M2 by MGIG. The classification system, the methodology for assessing ecological conditions, and the most relevant period for sample collection for the "phytobenthos" parameter have been developed and updated, and are currently adopted and already put in effort within Regulation No. H-4/2013 (last amended and complemented in SG. No. 85 of 02.10.2020 and SG No. 67 of 04.08.2023). The scientific contributions of her PhD thesis are also significant (Contributions 1 and 2): the study enriches the knowledge of the Bulgarian and regional algal floras, as well as of the distribution and ecology of species forming the diatom communities in some quite interesting habitats; 14 new species to the Bulgarian diatom flora were reported, whereas one very rare species was discovered for first time in Europe; a number of rare and/or endangered species were also recorded in the studied rivers.

# Evaluation of the quality of the scientific publications reflecting the studies in the doctoral thesis

Tsvetelina Isheva presents two publications reflecting her doctoral research, of which she is also the first author: one published in 2016 in Botanica Serbica (Q3, SJR 0.265), an international journal, and a second published in 2020 in Comptes Rendus de l'Academie Bulgare des Sciences (Q2, SJR 0.244, IF 0.378). The first publication has already been cited nine times. With the two presented publications in Q2 and Q3 journals, one of which is an international journal as well, Tsvetelina Isheva meets the current national requirements and the requirements of the BAS, including the specific requirements of IBER-BAS, for obtaining the educational and scientific degree "doctor" (PhD).

# Reasoned answer to the question to what extent the research in the PhD thesis is primarily the personal work of the doctoral student

I have been knowning Tsvetelina Isheva since the time when she was a newly graduate and a part-time assistant at St. Kliment Ohridski University of Sofia. I remember her for her passion for working with diatoms under the microscope, and for the energy and enthusiasm with which she taught students' practices. Based on my personal impressions and on my direct observations of the way she works, as well as on the basis of the materials provided for the defense of her PhD thesis, the way the results are presented in the thesis, and the design of the thesis itself, I can confidently state that the PhD thesis and the presented publications to it are mainly the personal work of Tsvetelina Isheva. The same is also evident from the plagiarism check report provided to us.

#### Summary of the PhD thesis with English abstract

The summary of the thesis contains a total of 38 pages, incl. title pages, one page of acknowledgments and one page abstract in English, and it is very neatly done. It follows the structure of the thesis and reflects well the main moments of the research work with illustrations and figures, the conclusions, and the contributions of the work. The publications related to the thesis are presented as well, including their citations, and the participations of Tsvetelina Isheva in scientific forums in relation to the doctoral thesis are also listed.

## **Reasoned conclusion**

The presented thesis by Tsvetelina Yasenova Isheva is a complete scientific work with original scientific contributions and significant contributions in applied science. The PhD student demonstrates very good knowledge on the topic of her work, she is excellently prepared methodologically, and she is able to interpret the results of her research. Based on the presented documents and PhD thesis, Tsvetelina Yasenova Isheva has acquired the necessary theoretical and practical knowledge in her scientific field. Therefore, I recommend awarding Tsvetelina Yasenova Isheva the scientific and educational degree "doctor" (PhD) in scientific specialty 01.06.11 "Hydrobiology".

May 22<sup>nd</sup> 2024

Assoc. Prof. Ralitsa Zidarova, PhD