REVIEW

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on PhD thesis (dissertation) for the Doctor of Philosophy (PhD) academic degree in the field of

higher education 4. "Natural Sciences, Mathematics and Informatics"; professional field 4.3. "Biological Sciences"; scientific specialty "Hydrobiology"

Reviewer: Dr. Yanka Nikolova Presolska, Associate Professor at the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences, Department of Aquatic Ecosystems, member of the scientific jury appointed by order No. 17/14.02.2025 of the Director of IBEI-BAS-Sofia.

Author of the dissertation: Galya Nikolaeva Georgieva-Mladenova

Topic: "CURRENT CHARACTERISTICS OF THE STATUS OF AQUATIC OLIGOCHAETAS AND ASSESSMENT OF THE INVASIVE POTENTIAL OF BRANCHIURA SOWERBYI BEDDARD, 1892 IN BULGARIAN SURFACE WATER BODIES", scientific supervision - Prof. Dr. Yordan Uzunov.

1. Biographical data

PhD student Galya Nikolaeva Georgieva-Mladenova was born in 1981 in Sofia. In 2008, she obtained a bachelor's degree in biology, and two years later she graduated as a Master of Environmental Biotechnology at the Faculty of Biology of Sofia University "KI. Ohridski". In the period 2013-2016, she was a full-time PhD student in the Department of Aquatic Ecosystems at the IBER-BAS. She was enrolled with the right to defend her thesis as of 01.01.2017 (Protocol of the Scientific council of the IBER-BAS No. 31/07.03.2017). Her thesis was discussed and approved for a defense by the Collegium of the Department of Aquatic Ecosystems, Protocol No. 14/17.01.2025.

Galya Georgieva-Mladenova joined the Department of Aquatic Ecosystems at the IBER-BAS at the end of 2011. After completing her doctoral studies, she was reassigned as a biologist, which position she currently holds. During this period, she has participated in over 20 research and applied projects and over 30 scientific forums in the country and abroad. She is a co-author of 20 scientific publications in peer-reviewed scientific journals and conference proceedings.

2. General characteristics of the PhD thesis - volume and structure

The dissertation is basically a systematic review of the diversity of the subclass Oligochaeta - one of the most widespread and diverse groups in the benthic fauna in various types of water bodies in our country. In addition to updating the data on the taxonomic composition of aquatic oligochaetes after 2007, the study also has the broad goal of analyzing their distribution and ecological preferences, and to assess the potential risk of invasion by the alien species *Branchiura sowerbyi* Beddard, 1892. The study of aquatic oligochaetes in Bulgaria has a long history. In this regard, the periodic systematization of current faunistic and ecological data, as we find in the present work, is an act of continuity and makes possible their successful use for scientific and applied purposes.

The dissertation is presented in the form of a book. It is well structured, with a total volume of 343 pages, including: 184 pages of main text and 143 pages of appendices. The main text includes Introduction – 1 page, Literature review – 25

The appendices are extremely informative. They are 10 in total and include results on the topic of the study – lists of the studied water bodies and points with indicated affiliation to an ecoregion, type of water body and date of sampling; list of aquatic oligochaetes established in Bulgaria with number of localities; their distribution in lake and river types; maps of the distribution of aquatic oligochaetes in Bulgaria (91); results of the dominance analysis and species similarity by watershed; values of the physicochemical indicators at which aquatic oligochaetes have been identified in Bulgaria; assessment of the invasive potential of *B. sowerbyi*.

The dissertation is well illustrated - a total of 19 tables and 61 figures illustrates the analyses and interpretation of the results. In terms of structure and volume, the dissertation fully corresponds to this type of scientific work. The abstract has been prepared in accordance with the requirements, it is presented in Bulgarian (with a summary in English) and adequately reflects the structure, content, scientific results and contributions of the main work.

3. Scientific papers on the PhD thesis research

The results of the study are published in three co-authored scientific articles, one of which is in *Acta zoologica bulgarica* (Q4), a specialized scientific publication indexed to WoS and falling within the quartile system, and the other two are reports printed in full text in Proceedings of materials from a foreign scientific event and an international scientific event held in our country. In all three publications, the doctoral student is the first and corresponding author. I consider this to be evidence of her leading role in the research, analysis of the results and scientific contributions in the published articles.

From the report for the assessment of the preparation under the credit system at the Bulgarian Academy of Sciences, it is clear that G. Georgieva covers the mandatory minimum of 130 points for Implementation of the educational program and exceeds that for Approbation of the implementation of the scientific program (64 points out of a mandatory minimum of 40 points) and Publications of scientific results on the topic of the dissertation (three publications and 140 points out of a mandatory minimum of two publications and 80 points).

4. Awareness and theoretical background of the candidate

The literature review is extensive and analytical. The origin, systematics and stages in the study of aquatic oligochaetes are reviewed comprehensively and in detail, as well as the problems and difficulties in the modern classification of some of the families. The author pays special attention to the taxonomic features used in the systematics of freshwater oligochaetes and the distribution of the group worldwide.

Faunistic studies of aquatic oligochaetes in Bulgaria are discussed in a separate subchapter of the literature review. The large number of cited sources, grouped and analyzed thematically demonstrate an exceptionally good knowledge not only of the chronology, but also of the scope of studies on this group in our country.

The report on research on the species *Branchiura sowerbyi* covers a wide range of studies with the idea of answering the question - is it an invasive species or not? They concern the general biological characteristics of the species, environmental factors that are important for its distribution and reproduction, data on the natural range and distribution around the world, as well as studies conducted in Bulgaria.

The presented literature review testifies to the very good awareness of the author on the topic. The list of used literature includes 239 titles, of which 22 are in Cyrillic (15 titles in Bulgarian

and seven in Russian), and the remaining 217 - in Latin (respectively 192 in English, 19 - in German, three - in Portuguese, two - in French and one - in Slovak).

The main goal of the study – to develop a modern generalized taxonomic overview of the diversity, distribution and ecology of aquatic oligochaetes in surface water bodies of Bulgaria, and to assess the invasive potential of the Asian aquatic oligochaete *Branchiura sowerbyi* Beddard,1892, logically determines the specific tasks set. They are related to updating the species composition of aquatic oligochaetes in Bulgaria, analysis of the distribution, frequency of occurrence and dominance of species by catchment areas, river basins, categories and types of freshwater bodies, characterization of the distribution of the identified species depending on the main environmental factors, as well as a study of the current distribution of the model object *Branchiura sowerbyi* (Tubificidae) and assessment of its invasive potential. The doctoral student also sets herself the task of creating an electronic and web-based repository of faunistic and ecological data, which will serve as an information basis for the dissertation.

5. Methodical approach

The study is based on an extremely rich material – a total of 929 collections from 434 sites at 262 freshwater bodies – rivers, lakes, dams and marshes (of which 167 lotic and 95 lentic ecosystems) from the three main watersheds in Bulgaria. In the collection and processing of the materials, established and generally accepted standardized methods for sampling and laboratory processing of benthic invertebrates were used. The taxonomic determination of the oligochaetes was carried out based on species-specific morphological characters with a motivated choice for the used classification of the species in the subclass Oligochaeta.

To fulfill the specific study tasks, G. Georgieva applies classical methodological approaches, some of which are correctly described. Such are the analysis of species and coenotic diversity (dominant analysis) according to De Vries (1937) and Kozhova (1970) and similarity of species composition according to Sörensen (1948). Statistical analyses were performed using multivariate statistical methods: Cluster analysis using the UPGMA method, correlation analysis for assessing the dependence between the abundance of the studied taxa as a function of environmental factors and ordination methods: analysis of the principal components (Principal Component Analysis, PCA), correlation analysis (Correspondence Analysis, CA) and MDS. In an applied aspect, the organized electronic database on the taxonomic diversity of aquatic oligochaetes in Bulgaria allows reliable analyses of data sets and logical interpretation of the results.

From the above it is clear that the author knows and successfully applies modern methods for ecological research. Her skillful handling of data in the processing and analysis of information from field observations, as well as from literary sources, undoubtedly proves her good methodological background.

6. Significance and persuasiveness of the obtained results, interpretations and conclusions.

The rich material based on which the faunistic and ecological analyses were carried out, as well as the use of modern methods for statistical data processing determine the reliability of the obtained results and the generalizations and conclusions. The results of the scientific research are presented in five subchapters, including: (i) faunistic analysis of aquatic oligochaetes in Bulgaria, incl. species composition and dominant analysis; (ii) their distribution by watersheds and types of surface water bodies; (iii) importance of main environmental factors for their

distribution in the country; (iv) ecological preferences and assessment of the invasive potential of *Branchiura sowerbyi* in Bulgaria; (v) presentation of the functionalities of the organized database. The results are analyzed in detail and well-illustrated with tables, graphs and diagrams. The skills of the doctoral student for data analysis and interpretation are also proven by the generalizations and conclusions made.

7. Nature of scientific contributions

The dissertation has several significant contributions of a chorological, faunistic and ecological nature, which upgrade the existing knowledge on the aquatic oligochaetes on a national and regional scale. Based on the research conducted and the results obtained, generalizations of an indisputable contribution have been drawn. In general, they could be summarized as follows:

Horological contributions: The rich material that the author analyzes allows her to present in detail the distribution of each species in Bulgaria with listed localities and collections. The maps in Appendix 4 are particularly valuable as a means of visualization.

Faunistic contributions: The rare species Piguetiella blanci (Piguet, 1906) is reported for the first time for the Bulgarian oligochaete fauna, found in two localities at once (Black Sea and Danube River catchment basins). In total 267 new localities of aquatic oligochaetes have been identified, which expands the knowledge of their distribution area in Bulgaria. New localities of rare species have been identified, with the species Rh. falciformis being registered for the first time in the Black Sea catchment basin. For the first time in our country, the species B. sowerby is reported from the brackish coastal lakes Atanasovsko Lake and Lake Mandra – Iztok. For the first time, a finding of an oligochaete species (B. sowerbyi) with teratological changes has been registered. Through a detailed faunal analysis, the distribution of oligochaetes in the main catchment areas of Bulgaria is clarified, establishing a commensurability between the number of species and the size of the catchments, resp. the diversity of habitats. In addition, the species that determine the appearance of the oligochaete communities in them have been highlighted. The species similarity by watershed has also been analyzed. For the first time, a systematic study of the Bulgarian oligochaete fauna from temporarily drying up water bodies has been carried out, in which the species composition and the dynamics of colonization of the newly flooded water bodies have been established. The summarized data from the dominant analysis of aquatic oligochaetes in Bulgaria show a predominance of the "small" (20) and "rare" (45) species, at the expense of the "mass" (2) and "common" (27) species of oligochaetes. The analysis of the distribution of B. sowerbyi places it in the category of the "common" species.

Ecological contributions: The presence of a large array of primary data on physical and chemical water parameters per sample enables the author to skillfully analyze the distribution of oligochaetes depending on various ecological factors and to derive valuable ecological generalizations for the studied group as a whole and for its individual representatives. When analyzing the data on the altitudinal distribution of oligochaetes in Bulgaria, a weak negative correlation was established between altitude and the dynamics of the number of aquatic oligochaetes. Despite the wide temperature range in which some species are established, the largest percentage of species is established between 20°C and 25°C, followed by that in the interval 15°-20°C. The author establishes the highest species richness at oxygen content in the water within the range of $6.01\div8$ mg/L O₂. The preference for slightly/weakly alkaline water environments is accompanied by a tendency to increase species richness with alkalization of the

environment. Regarding the ecological preferences of *B. sowerbyi*, the complex influence of environmental factors on the distribution of the species was analyzed using the principal component analysis (PCA) method, proving the determining role of the substrate type and the accumulated organic mass in the bottom sediments in this process.

From **a scientific and applied perspective**, I find the assessment of the invasive potential of *B. sowerbyi* valuable, made using two methods: the adapted version of the "Weed Risk Assessment" tool (compared to one developed for the Netherlands), and the risk assessment scale using the Risk Assessment Procedure (RAP) method. Both assessments report a low risk of the alien species *B. sowerbyi* becoming invasive on the territory of Bulgaria.

I positively evaluate the created electronic database of faunal, physical, hydrological and hydrochemical data on aquatic Oligochaeta in Bulgaria (with all its functionalities and upgrade options) and believe that it would be a useful tool in the future work of the author.

8. Critical notes and comments

I have several critical notes and comments on the dissertation in its final version, which, however, do not diminish its value. The more significant ones are:

- When analyzing the frequency of occurrence, you divide aquatic oligochaetes into four categories. Do you think the expression "small-numbered species" is appropriate in this case and if you had the opportunity to change it, what would you replace it with?

- The sentence "In the studied specimens from Bulgaria, spores of parasites were not found." (p. 31) is a result of the present study, therefore it has no place in the literature review.

- In the literature review of the studies on *Branchiura sowerbyi* (p. 37) and on p. 147 of the main text it is mentioned that "At the moment there are two systematized studies after 2012, and only one title is cited - Georgieva & Uzunov 2015.

- The chapter "Material and Methods" does not reflect the applied tool/method of data analysis on the ecological preferences of aquatic oligochaetes, namely - Descriptive statistics.

- There are semantic inaccuracies in the titles of some tables and the text under the figures, e.g. Table 1 (p. 40), Table 5 (p. 86), Fig. 31 (p. 128), Fig. 33 (p. 130), etc.

- Table 4 (p. 50) should be a figure, since it is intended for illustration, and does not present data in the context of the presentation.

- In the included "Index of names" the species and genus names should be in *italics* (pp. 198 and 199).

- In some of the summary results, the emphasis is on the contributing element, and it would be more correct to move them to the appropriate place or paraphrase them.

- The documents on the procedure lack information on the presence of citations of printed articles on the topic of the dissertation. Such a reference would give additional weight to the results of the study.

- Map No. 91 shows localities of the taxon *Potamothrix* sp. juv., including those in remote regions of the country, are they one and the same species?

There are also technical inaccuracies in the text of the dissertation, for example:

- Species names are not in *italics* (e.g. on p. 10., Tab. 15).

- On some pages the formatting of the text is broken (mainly around figures).

- In the chapter "Results and Discussion", item 1.1. Species composition, the maps are given as Appendix 3, but they are in Appendix 4.

- There are duplications in the numbering of some tables, e.g. Tab. 1 - it is present both in the text and in the appendices, etc.

9. Motivated answer to the question whether the dissertation research is primarily the author's personal work

Based on the materials provided for the PhD defense, as well as on my personal impressions of Galya Georgieva's work, not only during her doctoral studies, but also as a member of projects' teams within the Aquatic Ecosystems Department, I believe that this work is to a significant extent her personal work, regardless of the assistance and consultations of her scientific supervisor.

10. Conclusion

The PhD thesis of Galya Geogrieva-Mladenova contains significant and original scientific contributions in the field of faunistics and ecology of aquatic oligochaetes in Bulgaria. The research conducted is up to date, contains innovative elements and is based on targeted studies using modern methods of data collection and analysis. With the development of this dissertation work, the author has undoubtedly deepened her theoretical knowledge on the topic being developed, demonstrating qualities and skills for independent scientific research. I believe that the dissertation meets the requirements set out in the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations on the Conditions and Procedures for the Acquisition of Scientific Degrees and for the Holding of Academic Positions in IBER-BAS. Based on the above, I give a **POSITIVE ASSESSMENT** of the dissertation and propose to the members of the Scientific Jury for the procedure to award **Galya Georgieva-Mladenova** the educational and scientific degree "**DOCTOR**" in Hydrobiology.

09.05.2025

Reviewer:

(assoc. prof. Y. Presolska, PhD)