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REVIEW

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", to the Research Group "Biodiversity, communities and processes in freshwater ecosystems", Division "Biodiversity and functioning of freshwater ecosystems", Department "Aquatic ecosystems" of IBER-BAS, with scientific supervisor Assoc. Prof. Dr. Vladimir Vassilev Valchev and scientific consultant Prof. Dr. Rosen Todorov Tsonev

Author of the dissertation: BORISLAVA PETROVA GYOSHEVA, PhD student at the Department of Aquatic Ecosystems, Division of Biodiversity and functioning of freshwater ecosystems, IBER-BAS, Sofia, Bulgaria

Dissertation Topic: "COMPOSITION AND PHYTOSOCIOLOGICAL STRUCTURE OF MACROPHYTE COMMUNITIES IN DIFFERENT TYPES OF WATER BASINS IN THE WATERSHED OF THE BULGARIAN SECTION OF THE DANUBE RIVER"

Reviewer: Assoc. Prof. Dr. Nikolay Ilchev Velev, IBER-BAS; field of higher education "4. Natural Sciences, Mathematics and Informatics", professional field "4.3. Biological sciences", scientific specialty "Ecology and Ecosystems Conservation", appointed as a member of the scientific jury by order № 15/14.02.2025 of the Director of IBER-BAS.

1. Brief introduction of the candidate.

PhD student Borislava Petrova Gyosheva was born on 18.05.1985. In the period 2004-2009 she was a student at Sofia University "St. Kliment Ohridski", Faculty of Biology. She obtained her Bachelor's degree in Biology in 2009. In the period 2010-2011 she was a student at the same university, obtaining a Master's degree - "Master of Biology – phycology" in 2011. She was a full-time PhD student in the scientific specialty 01.06.11 "Hydrobiology" in the period 2014-2016 at the Department "Aquatic Ecosystems" at IBER-BAS with scientific supervisor Assoc. Prof. Dr. Vladimir Valchev (IBER-BAS) and scientific consultant Prof. Dr. Rosen Tsonev ("St. Kliment Ohridski" Sofia University). The dissertation thesis of Borislava Gyosheva has been approved for referral to defense at the Collegium of the Department of Aquatic Ecosystems, Protocol № 12/10.01.2025.

In the period 2009-2010 she was employed at the Institute of Botany, Division "Flora and Florogenesis", as a Biologist. From 2010 to date, she has been employed at the Institute of Biodiversity and Ecosystem Research (IBER-BAS) as a Biologist and Assistant Professor, including the period of PhD studies. She has professional experience as a visiting lecturer in practical exercises at the Faculty of Biology, "St. Kliment Ohridski" Sofia University (2014-2016) and at the Medical University of Sofia (2020).

She has 12 participations in scientific forums in the country and abroad and 18 participations in scientific research and applied projects. Author and co-author of eight scientific publications in peer-reviewed scientific journals and conference proceedings.

2. Documents submitted under the procedure.

The materials submitted by the PhD student Borislava Gyosheva comply with the Regulations on the conditions and procedure for the acquisition of scientific degrees and for the occupation of academic positions at the Institute of Biodiversity and Ecosystem Research at BAS, including the following mandatory documents:

A) Inventory of documents; B) Dissertation; C) Abstract; D) Statement of contributions of the dissertation; E) List of publications on the topic of the dissertation, as well as copies of the publications themselves; F) List of participation in scientific forums with papers and posters reflecting the research of the dissertation, as well as copies of the printed abstracts; G) List of citations of the works on the topic of the dissertation; H) Scientific CV, including a complete list of publications; I) Master's degree diploma - copy certified by the Scientific Secretary of IBER-BAS; J) Bachelor's degree diploma - copy certified by the Scientific Secretary of IBER-BAS; K) Minutes of the meeting that referred the dissertation for defense; L) Report on the study process and credits obtained, certified by the Scientific Secretary of IBER-BAS; M) Abstract of the dissertation in English, containing the title of the work and the main points in it.

3. General characteristics of the dissertation - volume and structure.

The dissertation of Borislava Gyosheva is presented as a book body with a total volume of 263 pages and contains the following parts: Title page on which is written the name of the educating organization, the name of the candidate, the dissertation topic, the academic position, the academic degree and the name of the supervisor and the scientific advisor, as well as the month and year of completion of the dissertation and its submission for disclosure of the defense procedure; Abbreviations used (1 p.); Introduction (2 p.); Aim, Objectives and Working Hypothesis (1 p.); Literature Review (15 p.); Study Area (15 p.); Methods (15 p.); Results and

Discussion (159 p.); Conclusions (2 p.); Contributions (2 p.); Bibliography (21 p.); Acknowledgements (2 p.); Appendices (18 p.). "List of macrophyte species identified by site", "Synoptic table of *Lemnetea, Potamogetonetea, Bidentetea, Phragmito-Magnocaricetea* class associations" and "Group definitions for classification of hydrophytic vegetation associations" are given as appendices. Fifty-six figures and 20 tables are presented with the text of the PhD thesis. The structure of the dissertation is standard, containing the obligatory parts described in the Regulations on the Conditions and Procedure for the Acquisition of Scientific Degrees and for the Occupation of Academic Positions at IBER-BAS.

4. Topic Relevance.

The study of plant diversity is of great benefit for both research and applied purposes. A good knowledge of the flora and vegetation is necessary for the conservation and maintenance of natural habitats and for the effective management of protected areas and sites.

So far, no comprehensive classification scheme of the syntaxa of aquatic vegetation in the country, defined by the floristic approach, is available. The vegetation of Bulgaria has not been sufficiently studied using the Braun-Blanquet method, which makes it very difficult to compare the results of studies from different parts of the country, as well as with those from other European countries.

Due to the insufficient knowledge of the biodiversity of wetlands and aquatic vegetation in particular, as well as due to the insufficient study of plant communities by the Braun-Blanquet method in Bulgaria, the topic of the PhD thesis is defined as extremely significant and up-to-date. I am sure that the dissertation is fully in line with current scientific challenges in Europe and in the country.

5. Literary awareness and theoretical background of the candidate.

The Literature Review chapter is comprehensive and well-structured, divided into seven parts: 1) Macrophytes - definition, general concepts and notes; 2) Danube flood terrace and its habitats; 3) Studies of macrophytes in the floodplain of the Danube river, influence of environmental factors on aquatic vegetation; 4) Studies on species and phytocoenotic composition of macrophyte vegetation in wetlands in the Danube floodplain terrace in Bulgaria; 5) Phytocoenotic studies of aquatic vegetation in Europe and the Danube flood terrace; 6) Macrophyte communities - an indicator of the ecological status of water bodies and the influence of eutrophication on aquatic plants; 7) Influence of hydromorphological features of water bodies and their connectivity with the main river on the macrophyte communities.

Literature sources are reviewed in a logical sequence. The literature review covers the whole period from the earliest studies on the flora and vegetation of wetlands in Bulgaria to the present day. All available literature data from the study area to date are summarized, including projects data. The literature review of studies on macrophyte vegetation is detailed and comprehensive, covering literature on both the dominant and floristic methodological approaches. It is concluded that there is a general lack of modern phytocoenotic studies of Danube wetlands in Bulgaria, which highlights the importance and relevance of the topic of this PhD thesis.

The presented literature review demonstrates the high level of awareness of the PhD student on the topic. A total of 312 sources of information, including normative documents - 72 in Cyrillic and 240 in Latin are cited in the dissertation. The PhD student is well acquainted with the floristic and phytocoenotic studies of the Danube floodplain terraces carried out so far.

6. Methodological approach.

Chapter "5. Methods" is very detailed. The methods for studying aquatic communities are divided into two separate subchapters - 5.1. Methods for studying macrophytes using the transect approach and Köhler's (1978) method, in the context of the Water Framework Directive and the assessment of the ecological status of waterbodies under the Biological Quality Elements – Macrophytes and 5.2. Methods for studying macrophytes using the classical Braun-Blanquet approach of phytocoenotic studies. The taxonomic status of all species was synchronized with the Euro+Med Plant Database. Ordination methods such as PCA (Principal Component Analysis), CCA (Canonical Correspondence Analysis), RDA (Redundancy Analysis), which are recognized and widely used in the field of numerical syntaxonomy, were applied. Software packages such as CANOCO (Ter Braak & Šmilauer, 2002) and R v. 4.4.2 (R Core Team, 2024) were used for the analyses. These are some of the most widely used in the field of phytocoenology. The "Cocktail Method", which works with formal definitions of vegetation types and logical operators, was used to define the syntaxa. Formal definitions for the associations were applied using the "New Cocktail Method ", which was developed by Landucci et. al. (2015). The method builds on the classical cocktail method of Bruelheide (1997, 2000) and can work with sociological and with functional groups of species, which is a novelty in phytocoenology. The analysis was performed with the software package JUICE 7 (Tichý, 2002). Functional species groups were defined and applied for classification of floristically poor vegetation types. They are used when clear and well-functioning sociological species groups cannot be defined. For the purpose of this PhD thesis, some modifications and additions to the expert file of Landucci et al. (2015, 2020) are proposed.

The field studies were conducted properly. They were carried out in the period 2014-2016. Twenty-six basins located in the floodplain of the Danube River and its tributaries were studied. Four Hundred Eighty-Two relevés were made according to the methodology of Braun-Blanquet (1964). In the species assessment, exact percentages are given, which is a modern trend in phytocoenology. Syntaxonomy and nomenclature are synchronized with the latest revision of the Vegetation of the Czech Republic (Sumberova et al., 2011), the hierarchical classification of the vegetation of Europe by Mucina et al. (2016) and the fourth edition of the International Code of Phytosociological Nomenclature, 4th edition - ICPN (Theurillat et al., 2021).

The application of a set of complementary classification methods contributes to the achievement of high confidence syntaxonomic decisions. I believe that the chosen research methods fully correspond to the set goal.

7. Significance and cogency of the results, interpretations and conclusions.

The results are presented in "Chapter 6. Results and Discussion". It is divided into two main parts - 6.1. Transect approach and method of Köhler (1978) and 6.2. Phytocoenotic study of aquatic vegetation, which parts logically follow the two methodological approaches. The material is described in great detail on 159 p. Fifty-five figures and 14 tables are appended to present the results and to aid discussion.

Regarding the flora, the species richness of aquatic plants in the studied ponds has been established. One hundred and fifteen taxa have been identified, representing about 30% of their total number in the country. Sixteen of the species are of conservation importance. Six species of alien invasive plants were recorded, incl. *Elodea nuttallii*, which is included in the list of the most dangerous invasive alien species threatening biodiversity in Europe. Standard indices for the representation of species diversity were calculated: species richness index (S), abundance index (N), Shannon-Weaver heterogeneity index (H'), Pielou index of evenness of species distribution in the coenoses.

In terms of syntaxonomy, 482 relevés of hydrophytic and helophytic vegetation were made. The identified syntaxa were classified into 4 classes, 7 orders, 11 alliances, 33 associations, 17 plant communities. Some associations are listed for the first time for Bulgaria. The similarity of the described helophytic and hydrophytic coenoses is represented by two dendrograms. A new formal definition has been developed for one of the associations identified. The environmental factors influencing the formation of the studied aquatic vegetation are analyzed. In interpreting the results, the PhD student demonstrated critical thinking and appropriate citation of relevant literature sources.

The way the results are presented is appropriate and has the necessary clarity. The results obtained objectively reflect the research conducted. The PhD student has presented his results in a logically ordered style, at a high scientific level and with correctly used scientific terminology. The results obtained are of significant importance in the field of flora and syntaxonomy of aquatic vegetation. In conclusion, I can say that a modern and significant study has been made in the field of aquatic vegetation, which is at a high scientific level and in line with the developments of leading European scientists in the field.

8. Critical notes to the dissertation.

I have no significant criticisms of the dissertation! Only some minor inaccuracies are found, which in no way compromise the results obtained and the high value of the PhD thesis:

- In several places in the thesis (pp. 47, 51, 52) the term "vegetation unit" from the English-language literature is translated incorrect in Bulgarian, which introduces some confusion. Depending on the context of the sentence, I recommend using more correct terms such as 'cluster', 'syntaxon' or 'vegetation type' when translating this term. Where possible, a specific syntaxonomic rank may also be used, such as association, alliance, etc.
- In Chapter 6.2. Phytocoenotic study of aquatic vegetation, p. 94, four associations are listed as described for the first time for Bulgaria, while five associations are listed in the contributions. It is good to synchronize this information.
- Further to the above remark, from the text about ass. *Spirodelo polyrhizae-Aldrovandetum vesiculosae*, it is not clear whether the association is new for Bulgaria or a new locality for Bulgaria is established (i.e. a new chorology), because the same is mentioned in the contributions.

9. Scientific contributions.

As a result of the conducted research, eight original scientific contributions and three confirmatory scientific contributions are presented in the dissertation of Borislava Gyosheva. All scientific contributions are significant. Two original scientific contributions can be pointed out as contributions of very high value: 1) the description of new associations for Bulgaria; and 2) the development of a new formal definition for the association *Ceratophyllo demersi-Elodeetum nuttallii*, which can be applied to the expert file of the "New Cocktail Method" of Landucci et al. (2015).

The scientific and applied contributions are: 1) the new scientific information obtained on the aquatic vegetation in Bulgaria, resulting from the analyses of the water quality assessment according to a widely applied methodology in Europe; and 2) the obtained data, resulting from the application of different approaches for the study of macrophyte vegetation and water bodies, are directly relevant for the conservation of rare and endangered species and phytocoenoses, for the conservation of the biodiversity of aquatic ecosystems in general, and also for the restoration of water quality.

I accept the statement of contributions submitted by the PhD student Borislava Gyosheva. The contributions reflect the results obtained from the dissertation work and have both scientific and applied significance. The scientific and scientific-applied contributions listed clearly show that the dissertation has scientific novelty.

10. Evaluation of the quality of the scientific papers reflecting the research on the dissertation.

The CV of the PhD student lists a total of eight collective publications in peer-reviewed journals and conference proceedings. Three of the publications are related to the dissertation and reflect parts of it, respectively. The PhD student is the first author of all three publications. Results of the dissertation have also been presented at four international scientific forums. The publications presented in the dissertation are in English, two of them are published in international journals, refereed and indexed in world-known databases of scientific information. One of the publications is in journal Ecohydrology & Hydrobiology of the Elsevier publishing house and have both Impact Factor (IF) and SCImago Journal Rank (SJR), as they are referenced and indexed in Web of Science and Scopus: JCR-IF (Web of Science) (2020): 3.215, Q2; SJR (Scopus): 0.531, Q2. Fifteen citations were identified for the same publication. The presence of 15 citations within four years only unambiguously indicates the high quality of the PhD student's scientific work.

11. A reasoned answer to the question to what extent the research on the dissertation is primarily the personal work of the PhD student

Having read in detail the materials presented for the defense, I am firmly convinced that Borislava Gyosheva is the main author of the dissertation. The results obtained, the contributions and conclusions drawn, as well as the publications related to the dissertation, also represent a personal contribution of the PhD student. Borislava Gyosheva is the first author of all three publications on the topic of the dissertation.

REASONED CONCLUSION:

On the basis of the various research methods learned and applied by the PhD student, the correctly derived experiments, the significant results obtained, as well as the generalizations and conclusions drawn, I believe that the submitted dissertation meets the requirements set forth 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, which gives me reason to rate the work **POSITIVE**.

I take the liberty to propose to the Honorable Scientific Jury also to vote **positively** and to award to biologist Borislava Petrova Gyosheva the educational and scientific degree **"Doctor"** in the scientific specialty "Hydrobiology".

Date: 26.03.2025 **REVIEWER:** .....

Sofia (Assoc. Prof. Dr. Nikolay Velev)