



REVIEW

on a dissertation work for obtaining the educational and scientific degree "doctor" by : field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.4 Earth sciences, scientific specialty Ecology and ecosystem protection.

Author of the dissertation: Radoslava Georgieva Zaharieva, full-time doctoral student at the Department of "Agroecology and Environmental Protection" at the Agricultural University, Plovdiv

Dissertation topic: Parasites and parasite communities of fish from the Danube River - ecology and biodiversity

Reviewer: Prof. Vladislav Haralampiev Popov, PhD, Agricultural University-Plovdiv, field of higher education 4. "Natural sciences, mathematics and informatics", professional direction 4.4 "Earth sciences", scientific specialty "Ecology and ecosystem protection".

designated as a member of the scientific jury by order No. RD-16/1118 of 31.10.2022 by the Rector of AU.

1. Brief introduction of the candidate

Candidate Radoslava Zaharieva was born in 1991 in the village of Branipole, Plovdiv region. She obtained a Bachelor's degree in "Agricultural Economics" in 2014, and subsequently in 2016 a Master's degree in "Ecology of rural systems" at the Agricultural University (AU)-Plovdiv. She enrolled as a full-time doctoral student at the Department of Agroecology and Environmental Protection at the AU in January 2019 in the scientific specialty "Ecology and ecosystem protection".

The applicant demonstrates a good level of the English language. My direct observations of the candidate show a good organization of the implementation of the scientific and applied tasks, a good ability to fulfill the duties and tasks on time, a responsible attitude to work, loyalty, and precision. Shows active participation in scientific conferences - Agriculture For Life, Life For Agriculture conference in Bucharest, Romania, June 3-5, 2021 with two scientific presentations and published abstracts (Web of Science), International May Conference on Strategic Management (IMCSM21) in Bor, Serbia, May 28-30, 2021 with 2 presentations and published abstracts (Web of Science), Agriculture For Life, Life For Agriculture conference in Bucharest, Romania, June 4-6, 2020 with two scientific presentations and published abstracts (Web of Science). She also participates in projects - Scientific research project No. 10-21, No. 05-20, and No. 03-19 "Parasites and parasite communities of fish from the Danube River - ecology and biodiversity" in the call "Support of doctoral programs" at the AU Centre for Scientific Research, as well as Project No. 17-12 in the direction "Support of the publication activity" by

financing three scientific publications at the Centre for Scientific Research at the AU-Plovdiv.

2. Relevance of the problem

The issues of the dissertation work are relevant, due to the insufficient data, regarding the indicators of invasion, the structure of the parasite communities, their seasonal changes, as well as the circulation paths of the helminth flow in the Danube River, incl. and in Bulgaria. Research on the helminth fauna of freshwater fish species from the Bulgarian section of the Danube River is mainly focused on the lower section of the river. Scientific studies on the parasites and parasite communities of freshwater fish species from the upper reaches of the river in Bulgaria are extremely few. Single helminthological studies of fish from the freshwater ecosystem of the Danube after the river entered Bulgarian territory date back only to the 1960s. Based on the scientific publications presented in the literature review, it can be seen that some of the fish species studied in this thesis (*Al. immaculata* , *B. ballerus* , *B. gymnotrachelus* , *C. elongata* , *C. taenia* , *P. cultratus* , *Rhodeus amarus* (Bloch, 1782), *S. bulgarica* , *V. vimba* and others) are among the understudied species of the Danube ichthyofauna. Two of the dominant fish species in this thesis (*Alb. alburnus* and *Ch. nasus*) are also poorly studied for helminths. No data were found for research on parasitic communities of *C. elongata*, *H. molitrix*, *P. cultratus*, *Rh. amarus*, *S. bulgarica*, *V. vimba* from the Danube River not only on the territory of Bulgaria, but also on the territory of other countries through which the river flows, as well as for its basin. There are single studies on the parasite communities of *Ch. nasus* from the Danube in other countries. All this gives rise to the interest and the need to carry out the dissertation work.

3. Purpose, tasks, hypotheses and research methods

The purpose of the scientific research, as well as the set scientific tasks for its achievement, are clear and well defined. They reflect the author's scientific approaches to test the scientific hypothesis, which implies the discovery and accumulation of new data, on the parasites and parasite communities of fishes of the Danube river freshwater ecosystem, and more specifically ecobiological studies on freshwater helminths and the dominant fish species of Danube River, comparative examination of the helminth biodiversity of the investigated fish species and the helminth communities of the dominant fish species of the Danube River and their seasonal changes. Research approaches are based on a well-conducted and extensive literature review and the identified gaps in data and information on the subject.

The "Materials and methods" section provides sufficiently detailed and accurate information about the complex nature of the research - suitable research sites, well-studied and described biotopes and the types of fish specimens (during the period 2019-2021, 2367 specimens belonging to 8 families were collected and 31 species of freshwater fish). Research methods are appropriate, as are field and laboratory research. The wide range of indicators for the study of biodiversity, the appropriately selected indicators indices for the study of the structure of parasite

communities, as well as modern methods of analysis and statistical processing of the data, strengthening the credibility of the obtained results and conclusions, are impressive. It must be concluded that the dissertation student has mastered the proposed standard research methodology to a good extent.

4. Transparency and presentation of the obtained results

In terms of volume and structure, the dissertation meets the requirements for a dissertation for the ONS "Doctor". The dissertation covers 250 pages and contains 108 tables and 75 figures. 206 literature sources were used, of which 45 in Cyrillic and 144 in Latin and 17 online databases. The dissertation includes all the required sections and subsections, which allows the tracking of the complex studies in their interrelationship. The dissertation is well illustrated with tables, figures and diagrams that support the perception of information, data and conclusions. Statistical treatment of the results and regression and correlation dependences are specifically and clearly presented. The dissertation uses a clear scholarly style that is both scientifically sound and comprehensible.

5. Discussion of results and used literature

The literature review is sufficiently detailed, systematic and meets the goal of conducting a complex study of the subject. It provides a detailed overview of studies on helminths of the type Plathelminths (classes Trematoda, Cestoda, Acanthocephala, Nematoda) and helminth communities of freshwater fish species from the Danube River and the river basin from the territory of Bulgaria and those by authors in other countries for the period from 1959 to 2022. The literature reference includes 101 scientific publications. Research related to the biology, ecology, and other aspects of helminthological studies of freshwater fishes in relation to the subject of this dissertation is indicated. The results of scientific research on helminths and helminth communities of freshwater fish from the Danube River and the Danube River Basin in different countries and those related to their study on the territory of Bulgaria have been tracked. The review provides a good basis for deducing the argumentation of the scientific study.

The dissertation student systematically and consistently presents the achieved results and the obtained data in the "Results and Discussion" section, making a well-founded discussion on them, following the sequence of the scientific tasks set for implementation. It shows the acquired experience of the dissertation student in analyzing the obtained scientific data and interpreting them. The study of the biodiversity of the investigated species of helminths covers 2367 specimens of fish belonging to 31 species, collected from 5 biotopes of the Danube River, during the period 2019-2021 an invasion was found with 20391 specimens of helminths belonging to 4 classes (Trematoda, Cestoda, Acanthocephala and Nematoda), 22 families, 27 genera and 34 species. For each of the established helminth species, synonyms, taxonomic position, hosts, localization, localities, occurrence, rank of invasion (minimum - maximum), season, brief data on the biology of the species (intermediate and final hosts) and notes on the species are recorded. The study of the biodiversity of helminths by hosts presents the distribution of the species of

helminths (Trematoda, Cestoda, Acanthocephala, Nematoda) established during the study by fish species. No helminths were detected in six of the 31 fish species examined (*L. gibbosus*, *C. taenia*, *B. ballerus*, *G. gobio*, *H. molitrix* and *R. amarus*). For the remaining 25 species of fish, data on their biology, ecology and distribution are given. The helminths and helminth communities of the dominant fish species in the ichthyocenoses of the freshwater ecosystem of the Danube River have been studied and described in detail, namely *Abr. brama*, *Alb. alburnus* and *Ch. nasus*. The three dominant fish species were selected as models for the analysis of helminth communities.

In the discussion of the obtained results, the doctoral student well interpreted the differences in the study of the helminth fauna of the scobar from the Danube River and its basin on the territory of other countries, including and for the Bulgarian section of the river, as well as from its basin in Bulgaria. The merit of the work is the detailed description of distribution of the helminths found in the present study in *Chondrostoma nasus* along the course of the Danube River and in its basin. The helminths of 22 non-dominant fish species are also described, with detailed data on the distribution of the helminths established in the present study in *Babka gymnotrachelus* along the course of the Danube River and in its basin. During an ecological-parasitological study of 31 species of fish (from 8 families), caught from 5 biotopes along the Danube River (biotopes Kudelin, Novo Selo, Yasen, Koshava and Kutovo), 34 species with 20,391 specimens of helminths were found. Established helminths belong to 4 classes. A comparative examination of the helminth communities of dominant fish species *Abr. brama*, *Alb. alburnus* and *Ch. Nasus* is presented.

The seasonal changes in the invasion indicators of the dominant fish species (*Abr. brama*, *Alb. alburnus*, *Ch. nasus*) were examined. The analysis of the component communities by season was based on the determination of the main indices of invasion (MI, MA and P%) for each endohelminth species. The section also includes such indicators, taking into account the species composition and diversity, as well as the quantitative structure of the endohelminth complexes by host species. Infracommunities are described by the indicators: total number of species; total number of copies; average number of species; average number of specimens; diversity of faunal complexes represented by the Brillouin index (HB), the Pielou evenness index (E) and the Simpson dominance index (C). In the dissertation, the seasonal changes of the helminth communities are presented by fish species.

The conclusions of the dissertation are related to the implementation of the set tasks, as they are argued in detail and lead to justified conclusions - for example, there was an infestation with 20391 specimens of helminths, belonging to 4 classes (Trematoda, Cestoda, Acanthocephala and Nematoda), 22 families, 27 genera and 34 species. 9 species of the class Trematoda were isolated, 8 species of class Cestoda, 4 species from the class Acanthocephala and 13 species from the class Nematoda. Kudelin, Yasen, Novo Selo, Koshava and Kutovo biotopes are new habitats for all established helminth species. *Sch. acheilognathi* is a new taxon for the helminth fauna and helminth communities of freshwater fish from the Danube River in Bulgaria. Six species of helminths are new to the Danube River and the

river basin in Bulgaria . Three types of helminths are new for the Danube River and the river basin. For 25 types of helminths, new hosts have been established in Bulgaria. Of the 31 fish species studied from 5 biotopes, the highest number of helminth species was found in *Abr. brama* (15 species), *V. vimba* (13 species) and *Ch. nasus* (11 species). The highest Brillouin diversity index was found for *Abr. brama* (HB=1.54); highest Pielou evenness index – at *Alb. alburnus* (E=0.85) and the highest Simpson dominance index – at *Ch. nasus* (C=0.99) in the spring season. The invasion parameters at *Abr. brama* show a maximum in spring, decreases in summer and a second peak in autumn ($\chi^2=31.18$, df=12, $p=0.002<0.05$); at *Alb. alburnus* – maximum in autumn, decreases in summer and a second peak in spring ($\chi^2=47.46$, df=12, $p=0.0000$). The helminth complexes of *Ch. nasus* (by P%) show a maximum in spring ($\chi^2=63.68$, df=32, $p=0.0007<0.05$), best expressed in the main species *Contracaecum* sp., where a decrease in summer and a second peak in autumn are found. Seasonal dynamics in species distribution and invasion parameters were found in *Abr. brama* and *Ch. nasus* . Seasonal dependence in *Alb. alburnus* was found only in the distribution of *N. skrjabini* and *Sph. Brama*.

Based on the above, the dissertation offers specific and appropriate recommendations.

6. Contributions of the dissertation work

The overall study presents the following scientific and scientific-applied contributions, with which I agree:

Scientific contributions

The scientific literature on research on parasites and parasite communities of fish from the freshwater ecosystem of the Danube River has been enriched.

The data on the species composition of the helminths of 25 species of fish from the Danube River have been enriched.

New data are provided on the invasion indicators of parasites pathogenic to the fish species studied - *Sch. acheilognathi* , *P. laevis* , *Contracaecum* sp., *E. excisus* and *R. acus* .

New data are provided on the invasion indicators of human pathogenic parasites - *Contracaecum* sp. and *E. excisus* .

Scientific and applied contributions

A new species of helminth has been identified for the helminth fauna and helminth communities of freshwater fish from the Bulgarian section of the Danube River (*Sch. acheilognathi*).

6 species (*L. confusus*, *Sph. bramae*, *N. cheilancristrotus* (larvae), *C. lacustris*, *Ph. obturans*, *K. intestinalis*) of new endohelminths were identified for the Danube River and the river basin in Bulgaria.

3 species (*L. confusus*, *N. cheilancristrotus* (larvae), *Ph. obturans*) of new endohelminths for the Danube River and the river basin have been identified.

For 25 species of helminths, new hosts have been established in Bulgaria. For 29 species of helminths, new hosts have been established for the Danube River and the river basin in Bulgaria. For 26 species of helminths, new hosts have been established for the Danube River and the river basin.

22 species of new helminth hosts have been identified for the Danube River and the river basin, including Bulgaria, as well as 18 species of new hosts of helminths in Bulgaria.

For the first time, the helminth communities of *Ch. nasus* from the Bulgarian section of the Danube River and in Bulgaria have been researched. The data on the helminth communities from the Danube river - *Alb. alburnus* and *Abr. brama* have been updated.

For the first time, the helminth communities of *Abr. brama*, *Alb. alburnus* and *Ch. nasus* from the Bulgarian section of the Danube, Kudelin biotope.

For the first time, seasonal differences in the helminth assemblages of *Abr. brama*, *Alb. alburnus* and *Ch. nasus* from the Danube river, Kudelin biotope have been determined.

Based on the contributions, **recommendations** were formulated related to the consumption of dominant fish species due to the presence of parasites, as well as permanent monitoring of invasion indicators with *Sch. acheilognathi*, *P. laevis*, *Contracaecum* sp., *E. excisus*, *R. acus* as pathogenic for freshwater fish with a view to reducing fish mortality.

7. Critical Notes and Questions

1. It is recommended that the contributions be more clearly and concretely differentiated into scientific and scientific-applied ones, because they are not presented in this way in the dissertation and the abstract.
2. It is important to better specify the practical utilization of research and the direction of recommendations, such as on invasive species monitoring and invasion indicators by the EEA authorities, and the focus of ecoparasitological and freshwater fish studies to track parasites and parasite complexes with a view to protecting the biological diversity of fish and fish resources.
3. Studies leading to clearer conclusions on the interrelationships between seasonal fluctuations and dynamics of established parasite species, biodiversity indices, invasion parameters and ecological or anthropogenic causes could also be deepened.
4. A very large number of biodiversity indicators were studied, as well as those concerning the comparative examination of the helminth biodiversity of the studied fish species and the helminth communities of the dominant fish species from the Danube River and their seasonal changes. Relevant conclusions, based on comparative analyzes of the obtained data, could be formulated by using statistical tools as well, such as multivariate analysis of variance (MANOVA), which will convey statistical significance of the differences found by biotopes, parasite species and communities, seasons, etc.

8. Published articles and citations

1. **Zaharieva R., Kirin D., 2020** . New data on parasites and parasite communities of *Alburnus alburnus* (Linnaeus, 1758) from the Danube River. Book of Proceedings, Scientific Papers. Series D. Animal Science. LXIII(2), 397-404. ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750 http://animalsciencejournal.usamv.ro/pdf/2020/issue_2/Art61.pdf
2. **Zaharieva R., Kirin D., 2020** . Parasites and parasite communities of the Common nase (*Chondrostoma nasus* (Linnaeus, 1758) from the Danube River. Book of Proceedings,

Scientific Papers. Series D. Animal Science. LXIII(2), 413-420. ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750, http://animalsciencejournal.usamv.ro/pdf/2020/issue_2/Art63.pdf

The presented abstract reflects objectively the structure and content of the dissertation work.

CONCLUSION:

Based on the various research methods learned and applied by the doctoral student, the correctly conducted experiments, the generalizations and conclusions made, I believe that the presented dissertation meets the requirements of the Law on the Development of the Academic Staff in Bulgaria and the Regulations of the Agricultural University-Plovdiv for its application, which gives me the reason to rate it **POSITIVELY**.

I take the liberty of proposing to the honorable Scientific Jury to also vote positively and award Radoslava Zaharieva the educational and scientific degree "**Doctor**" in the scientific specialty "Ecology and Ecosystem Protection".

Date: 10.11.2022
Plovdiv

REVIEWER:
(Prof. Dr. Vladislav Popov)