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R E V I E W

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of

Dissertation thesis: "Taxonomic revision of the genus *Gyrodactylus* (Monopisthocotyla: Gyrodactylidae) in freshwater fish in Bulgaria" under the procedure for acquiring an educational and scientific degree "Doctor" in professional field 4.3. Biological sciences

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1. General characteristics of the dissertation

The submitted set of materials includes the required documents and complies with the requirements specified in the Higher Education Act and the conditions and procedures for acquiring scientific degrees and occupying academic positions at the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences.
The dissertation is structured as follows: Introduction, Literature review, Aim and objectives, Conclusion, Findings, Contributions, Bibliography. The sections "Materials and Methods" and "Results and Discussion" do not exist as separate sections in body text, but are included as parts in three scientific publications. The "Conclusion" section provides a brief overview and summary of the most important results. The Conclusions are also presented in separate chapters, as are the Contributions. The list of references (Bibliography) contains 47 titles, 31 of which are in Latin and 16 in Cyrillic.
The abstract meets the generally accepted requirements, is presented in 24 pages, and summarizes the main points of the dissertation.

2. Literary awareness and theoretical background of the candidate

The literature review is focused, analytical, and concludes with a discussion that justifies

the need to develop the chosen dissertation topic. The literature review is sufficiently informative—it presents the issues thoroughly researched by the doctoral student: the study of the species of flatworms belonging to the genus *Gyrodactilus*, parasitizing fish from water basins in Bulgaria. The PhD student describes in detail the studies published in the 1970s and 1980s, a period marked by intensive research on this group in Bulgaria. The results of all other studies conducted to date on the genus *Gyrodactilus*, both in Bulgaria and in neighbouring countries, are also described. The literature review shows that the initial focus of research in Bulgaria was on *Gyrodactylus* parasites affecting fish of economic importance for both commercial and recreational fishing. This is understandable, as the disease caused by these flatworms – gyrodactylosis – is associated with damage to the skin, gills, and fins of fish caused by piercing, grinding, and eating away at the epidermis, which often leads to secondary bacterial and fungal infections, often resulting in the death of the host.

Today, cases of gyrodactylosis are a common problem in both carp and trout fish farms, which determines the relevance and importance of studying the species diversity of gyrodactylids in Bulgaria. The doctoral student has described all host fish species, the locations where they were caught, and the scope of the study of gyrodactylid fauna – whether it is only taxonomic or also includes data on the occurrence and intensity of parasitism. As a result of the analysis of all available sources, the doctoral student found that 36 species of the genus *Gyrodactilus* have been described in Bulgaria, parasitizing 37 fish species collected from 57 sites.

The literature review shows that although there is abundant information on the genus *Gyrodactylus* in Bulgarian waters, it has not been brought into line with current data on the taxonomy of the group. For most of the identified species, there are no taxonomic illustrations or photographs, nor biometric data on the distinguishing features that could serve as evidence. The PhD student correctly justifies the need to include new research tools in the methodological arsenal, such as molecular genetic analysis, which could lead to a possible reassessment of the taxonomic determination of already described species or assist in the description of new species.

The aim of the dissertation is well formulated and logically follows from the literature review and the identified problems in the taxonomic determination of representatives of the genus *Gyrodactilu*s parasitizing fish in Bulgarian waters.

3. Methodological approach

To characterize the species of the genus *Gyrodactylus* from freshwater fish in Bulgaria, materials from the Helminthological Collection of the Institute of Biodiversity and Ecosystem Research (IBER) of the Bulgarian Academy of Sciences (BAS) were used, collected by previous authors, as well as newly collected material by the doctoral student. The revised material contained in the collection includes 296 permanent microscopic preparations made between 1962 and 1996. The doctoral student collected her own material from two standing water bodies – Lake Srebarna and Dragoman Marsh, as well as from three rivers – Glazne, Malak Iskar, and Byala Reka. The fish from these water bodies were subjected to a complete parasitological analysis, with their skin, fins, gills, and nostrils being examined. The gyrodactylids found were used to prepare permanent specimens for morphological analysis, and parts of them were preserved for analysis of target DNA segments for species identification.

The species characterization is based on the biometrics of various structures of the gyrodactylus body, with detailed taxonomic illustrations of the hard parts of the attachment disc of each of the flatworm species contained in the helminthological collection of the Institute of Biodiversity and Ecosystem Research - BAS.

The methods used are adequate for the stated goal and tasks. The methodologies are described in detail in the three articles that make up the dissertation. They include morphological, biometric (mainly article 1) and molecular-genetic methods (DNA extraction from haptors, amplification and sequencing of a section of ribosomal DNA, statistical analysis) – articles 2 and 3.

4.Significance and credibility of the results obtained, interpretations, and conclusions

The dissertation presents a detailed morphological and morphometric analysis of 325 individuals of the genus *Gyrodactylus* from the Helminthological Collection of the Institute of Biology at the Bulgarian Academy of Sciences and 30 specimens of 6 species of gyrodactylus collected by the author between 2017 and 2022 from various species of freshwater fish. The results are presented clearly and systematically, well visualized with taxonomic drawings and tabulated data from morphological analysis. In interpreting the data obtained from the morphometric analysis, the doctoral student supports her arguments with a significant volume of literature sources. The conclusions are logically sound and convincingly defended. The author convincingly confirms the presence of 22 species of

gyrodactylids in the Bulgarian parasitofauna*: G. aphyae, G. cyprini, G. fossilis, G. gobii, G. gracilihamatus, G. katharineri, G. laevis, G. latus, G. leucisci, G. luciopercae, G. macrocornis, G. macronychus, G. malmbergi, G. markakulensis, G. medius, G. prostae, G. rhodei, G. shulmani, G. sprostonae, G. stankovici, G. truttae,* and *G. vimbi*.

She revised the collection materials and reclassified some species as defined by Kakacheva-Avramova (1976, 1977) as *G. longiradix* from *Gymnocephalus cernua*, the doctoral student reclassified as *G. luciopercae*. The gyrodactylids identified as *G. latus* (on the fish *Cobitis taenia*) are redefined as *G. papernai*. Some of the individuals reported as G. gobii from the host Gobio gobio are redefined as *G. dykovae* and *G. gobiensis*. Based on newly collected material, the doctoral student reports three new species for Bulgaria: G. *cobitis, G. melas,* and *G. perccotti*, the latter two being specific parasites of alien fish species that have been introduced into water basins in Bulgaria over the last decade.

5.Critical comments on the dissertation

I have no critical comments on the materials presented in the dissertation as a whole. However, the lack of justification in Article 1 for the inclusion of additional host species in the study somehow remains outside the scope of the study – a revision of the identification of species of the genus *Gyrodactylus* from the Helminthological Collection of the Institute of Biology at the Bulgarian Academy of Sciences, namely the two species of loaches (*Barbus petenyi* and *B. cyclolepis*) and the common gudgeon (*Gobio gobio*), raises some questions. Were the species included because of the unclear parasitofauna on them, or was the aim to expand the number of water basins from which *Gyrodactylus* specimens are collected? I cannot agree with the author that species of the genus *Barbus* or *G. gobio* are poorly studied, based on the information presented in the literature review and Table 1 of Article 1. If fish originating from unexplored rivers were sought, why specifically the Glazne River (Pirin mountain), the Malak Iskar River (Stara Planina mountain), and the Byala River (Eastern Rhodopes mountain)? There is justification for the inclusion of the invasive alien species Chinese minnow and Pseudorasbora. I recommend that in future publications, when writing the name of the Atlantic (Balkan) trout, the author adhere to the binomial structure of fish names, i.e. *Salmo trutta*.

6.Scientific contributions

The contributions in Nina Vancheva's dissertation are preceded by seven conclusions that adequately reflect the analysis of the results obtained by the doctoral student. I would only disagree with the wording of conclusion No. 5, which is more of an assumption based on the fact that a significant proportion of species of the genus *Gyrodactilus* parasitize a specific host, and it would be logical that when studying new fish species, they would turn out to be carriers of *Gyrodactylus* species not yet described in our country. I would also add my disagreement with the definition that most of the hosts studied are "economically important fish." On the contrary, apart from carp, silver carp, and bighead carp, and to some extent crucian carp, none of the species studied are of economic importance, i.e., they are not farmed in aquaculture and are not traded commercially. Conclusion No. 7 should be limited to its first part, because the impact of most gyrodactylids on the local biota has not been studied.

The author presents **eight contributions** from his research. Seven of the contributions are original and one is confirmatory in nature. The contributions are clearly formulated, argued on the basis of a significant amount of morphological, morphometric, and molecular-genetic data, and demonstrate both theoretical validity and practical value. The confirmation of already described species based on specimens from the helminthological collection of the Institute of Biology at the Bulgarian Academy of Sciences is supplemented by morphometric data and scientific illustrations. Three species new to the fauna of Bulgaria are described - *G. dykovae, G. gobiensis* and *G. papernai*, also from specimens from the helminthological collection.

No less significant, in my opinion, are contributions Nos. 4, 5, and 6, describing the first establishment in the fauna of Bulgaria of the species *G. perccotti* – a specific parasite of the invasive alien fish species *Perccottus glenii, G. melas* – a parasite of the invasive alien species of catfish *Ameiurus melas*, and *G. cobitis* – a parasite of loaches (Cobitidae).

A contribution of scientific and practical significance is the deposit of DNA fragments ITS-5.8S-ITS2 from the species *G. melas* and *G. perccotti*. The sequences have been deposited in GenBank, with fragments of 18S rDNA and a partial fragment of the mitochondrial COI gene also deposited for G. melas.

7.Assessment of the quality of scientific papers reflecting the research in the dissertation

Three publications are related to the topic of the dissertation, and in all three, PhD student Nina Vancheva is listed first among the authors. A common theme in the content of the publications is the study of representatives of the genus *Gyrodactilus*. The high quality of the articles can be assessed on the basis of the fact that all three have been published in specialized parasitology journals – *Parasitologia, Parasitology Research, and Acta Parasitologica*, with quartiles Q2, Q1, and Q3, respectively.

The citations of Nina Vancheva's published studies are proof of their significance. The total number of citations observed is 8. The results of the dissertation have also been presented at two scientific forums.

8.A reasoned answer to the question of the extent to which the research in the dissertation is primarily the work of the doctoral student

The doctoral student has worked in the field of flatworm taxonomy, combining different approaches. For the revision of the existing specimens in the helminthological collection, she used morphological characteristics and morphometric data to describe the individual species of the genus *Gyrodactilus*. Each description is accompanied by a scientific illustration of the hard parts of the attachment disc of gyrodactylids, ensuring transparency in taxonomy and providing evidence. The volume of measurements performed undoubtedly speaks of considerable experience, enabling independent work and personal interpretation of the results. The doctoral student uses molecular genetic analyses, which represent a significantly smaller part of the study as a whole, for only two of the gyrodactylus species (*G. perccotti* and *G. melas*) – species parasitizing on two species foreign to the Bulgarian ichthyofauna – Perccottus glenii and Ameiurus melas. In these publications, she works with a larger team of authors, but her leading role in both cases reflects her essential contribution to this type of research.

Undoubtedly, her scientific supervisor and scientific consultant have played a key role in her development as a researcher, working with both classical and new, promising methods in the field of taxonomic research. I have every reason to believe that the main research, analysis, and interpretations are the work of the doctoral student.

9.Conclusion

Nina Vancheva has presented to the Scientific Jury a dissertation that can be classified as a complete scientific work meeting the legal requirements for obtaining the educational and scientific degree of "doctor." The dissertation is clearly focused on specific problems related to the taxonomic affiliation of species of the genus *Gyrodactilus*. A sufficient amount of experimental material has been processed and analyzed, and a comprehensive approach has been applied, including a diverse methodological arsenal. The results obtained are original, and the proposed scientific contributions are significant. Based on the above and in accordance with the Law on the Development of Academic Staff in the Republic of Bulgaria, as well as the specific requirements of the Regulations for the Application of the ZRAS of the IBES-BAS for the acquisition of scientific degrees and academic positions, I propose that the esteemed Scientific Jury admit Nina Vancheva to the defence and award her the educational and scientific degree of " Doctor of Philosophy"

14.12.2025 **Prof. Eliza Uzunova, PhD**