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REVIEW

by

Prof Dr Yasen Mutafchiev, IBER-BAS, for the award of the educational and scientific degree “Doctor” in the doctoral program “Parasitology and Helminthology”, professional field “4.3. Biological Sciences”

About material submitted for the defence of PhD thesis: "Taxonomic revision of the genus Gyrodactylus (Monopisthocotyla: Gyrodactylidae) in freshwater fish in Bulgaria",

Author: Nina Vencheva Vancheva;

Scientific supervisor: Prof Dr Boyko B. Georgiev

Scientific consultant: Chief Assist Prof Dr Anelia B. Bobeva

Nina Vancheva received her Bachelor's degree in Biology in 2013 from the Faculty of Biology at Sofia University "St. Kliment Ohridski" and completed her Master's degree in Parasitology at the same university in 2015. From 2016 to 2019, she was a full-time PhD student at the Institute of Biodiversity and Ecosystem Studies, Bulgarian Academy of Sciences (IBES-BAS). Since 2020, she has been employed as a biologist in the Department of Animal Diversity and Resources at IBES-BAS.

The materials submitted by Nina Vancheva for her PhD defense comply with the *Regulations on the Conditions and Procedure for Acquiring the Scientific Degree "Doctor"* at IBES-BAS and include the following: List of documents; Dissertation; Abstract; Contributions of the dissertation; List of publications on the topic of the dissertation and copies of the publications themselves; List of participations in scientific forums with reports; List of citations of works on the topic of the dissertation; Scientific autobiography; Diploma for Master’s degree; Diploma for Bachelor's degree; Protocol of the meeting approving the dissertation for defence; Records of the educational activities and obtained credits; English summary of the dissertation.

General structure of the dissertation

The dissertation is prepared as a compilation of peer-reviewed scientific publications in English, accompanied by text in Bulgarian, totaling 89 pages. Its structure adheres to the requirements of the the Regulations on the conditions and procedure for acquiring scientific degrees and for occupying academic positions at IBER-BAS, 10 chapters are formed: Introduction, Literature review, Aim and objectives, Publications (3 scientific articles), Summary of the results, Declaration of originality,

Conclusions, Contributions, Literature, Acknowledgements. The literature cited in the general sections of the dissertation included of 16 titles in Cyrillic and 31 in Latin.

Literature awareness and theoretical knowledge of the Candidate

The Introduction outlines a detailed overview of the genus *Gyrodactylus*, emphasizing species diversity, host specificity, and the genus's importance as pathogens of fish in aquaculture and natural ecosystems. The economic importance of the *Gyrodactylus* spp. for the fishing industry also determines the significant interest in the group. Due to the high species diversity and limited morphologically informative features, molecular methods have become an integral part of taxonomic studies in the genus.

The Literature Review provides a detailed overview of *Gyrodactylus* reports in Bulgaria, identifying 36 representatives from 37 species of freshwater fish. Most data derive from studies conducted between the 1960s and late 1980s. A critical analysis indicates that previous contributions were primarily faunistic and do not allow thorough evaluation, with modern research on the genus in Bulgaria being sporadic. This highlights the relevance of the dissertation topic: characterizing the species composition, distribution, and host specificity of *Gyrodactylus* species from Bulgarian freshwater fish.

Methodological approach

The dissertation sets three tasks to update knowledge on the genus: Task 1 involves revising *Gyrodactylus* materials collected by previous authors and deposited in the Helminthological Collection of IBER-BAS and elaboration of contemporary morphological descriptions. Tasks 2 and 3 involve morphological and molecular studies of newly collected material, respectively. Morphometric indices and the choice of the ITS1–5.8S–ITS2 ribosomal DNA barcode region align with current taxonomic methods, demonstrating the candidate's thorough preparation.

Significance and persuasiveness of the obtained results, interpretations and conclusions

A total of 296 microscope slides available in the institute's collection containing 325 specimens of the genus *Gyrodactylus* collected in the period 1962 and 1996 were re-examined. Additionally, 30 specimens of 6 species collected by the PhD Candidate in the period 2017–2023 were studied. As a result, 21 previously reported species were confirmed; the identifications of two species reported for the fauna of Bulgaria were corrected, namely *G. longiradix* reidentified as *G. luciopercae*, and *G. latus* – as *G. papernai*; the species *G. latus* was however confirmed for the country from newly collected material. The monogeneans *G. dykovae*, *G. gobiensis*, *G. papernai* and *G. cobitis* are reported for the first time from native fish in Bulgaria. Two invasive species of the genus *Gyrodactylus* were also identified: *G. perccotti* a specific parasite of the invasive fish *Perccottus glenii*, and *G. melas* a parasite

of the invasive fish *Ameiurus melas*. In addition, the monogenean *Ligictaluridus pricei* (Ancyrocephalidae) was reported for the country also as a parasite of *A. melas*. All identifications were supported with detailed morphological descriptions, well-prepared illustrations and comparative tables.

DNA sequences for the entire ITS-5.8S-ITS2 region of five species - *G. fossilis*, *G. latus*, *G. malmbergi*, *G. melas* and *G. perccotti* were obtained.

The obtained results are significant, original and fulfil the aim and tasks set of the dissertation.

Critical notes on the dissertation work

The Literature Review could benefit from a summary of fish hosts studied in Bulgaria and an analysis of under-studied species and habitats, which are relevant for planning Task 2. Information about this is presented as conclusion 5 “In the collection materials, species of the genus *Gyrodactylus* predominate from economically important fish species such as *Cyprinus carpio*, *Carassius carassius*, *Squalius cephalus* and *Salmo trutta*, which have been studied extensively. Smaller fish species of no economic importance, have been studied less and it can be expected that additional species of *Gyrodactylus* will be reported from them”.

Task 2 (“Collection of new material and supplementing information on the morphology of species with modern approaches”) lacks clarification on what constitutes “modern approaches.” The dissertation’s stated contributions could be more detailed; for example, the number and identity of fish species studied for monogeneans should be specified.

Some discrepancies exist between the Abstract and the dissertation: the dissertation totals 89 pages, not 96; new material was collected from 2017–2023, not 2017–2024; there is only one table, not two; Table 1 represents a parasite-host list, not newly collected material; and the literature contains 16 Cyrillic references, not 17.

Nature of scientific contributions

The doctoral Candidate has formulated eight contributions, which I accept as original.

In summary

The dissertation represents an original scientific contribution conducted using modern methods and meets the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, as well as the Regulations of the Institute of Biodiversity and Biodiversity of the Bulgarian Academy of Sciences for the Acquisition of the

Educational and Scientific Degree "Doctor”. The minor critical notes and technical inconsistencies do not affect the results, their interpretation, or the conclusions.

I have known Nina Vancheva since her Master's thesis work in the research group “Taxonomy, Evolution, and Ecology of Helminths” and can confirm that the dissertation results are her original work. Her theoretical and methodological training provides a solid foundation for her future development as an independent scientist.

Based on the above, I propose that the esteemed jury award the educational and scientific degree "Doctor" to Nina Ventcheva Vancheva in the doctoral program "Parasitology and Helminthology," professional field "4.3. Biological Sciences."

Sofia, 19.12.2025