**PhD Thesis Acceptance Report**

**Research Discipline Council of Biological Sciences**

**Jagiellonian University in Kraków**

**Candidate's name and surname**: Pranav Unnikrishnan

**PhD Thesis Title**: Maintenance of *6Pgdh* Polymorphism in Bulb Mites: Insights from Field Studies, Experimental Evolution, and Life-History Trade-Offs

**Thesis Supervisor**: prof. dr hab. Wiesław Babik

**Assistant Supervisor / Second Supervisor/ Co-supervisor (if applicable)**: dr Agata Plesnar-Bielak

**Reviewer**: dr hab. Magdalena Witek

**1. The topic of the dissertation**

Research on genetic polymorphism has a long tradition and it has been conducted quite intensively over the past few decades. The doctoral dissertation presented by MSc Pranav Unnikrishnan aligns well with current research trends in this field, particularly those exploring the influence of ecological factors on the maintenance of genetic variability. The research topic undertaken in the dissertation is significant, as it concentrates on potential environmental and evolutionary factors that may directly shape the persistence of genetic variation in natural populations. As a research model, the author chose important metabolic genes—*6Pgdh* in bulb mites. The doctoral Candidate demonstrated that environmental variability may be a significant factor influencing the maintenance of balanced genetic polymorphism. Furthermore, using an experimental evolution approach, MSc Pranav Unnikrishnan showed that the interaction between abiotic factors (such as temperature) and biotic factors (such as the intensity of sexual selection) can play a crucial role in maintaining *6Pgdh* gene polymorphism.
To conclude, the dissertation is relevant to the development of the discipline of biological sciences.

**2. The candidate's knowledge**

The dissertation consists of five main chapters, including a general introduction and conclusion. The results of the remaining three chapters have been published as scientific article (Chapters 1 and 3) and as a preprint (Chapter 2). The doctoral dissertation demonstrates the Candidate's good knowledge of the topic related to genetic polymorphism in general and to *6Pgdh* polymorphism in bulb mites. In my opinion, the general introduction is particularly well-written, guiding the reader clearly and engagingly

from broader evolutionary questions to the specific issue of genetic polymorphism in *6Pgdh* genes in bulb mites. Both the general introduction and the discussions within individual chapters are well-grounded in the existing body of knowledge. They are properly supported by literature, including both historical sources and many recent studies, which effectively introduce and summarize the research presented in the dissertation. To conclude, the dissertation demonstrates a broad general theoretical knowledge of Candidate in the discipline of the biological sciences.

**3. Independence of the candidate**

The doctoral dissertation does not provide sufficiently detailed information regarding the Candidate's specific role and contributions at the various stages of the research, however, such details are available in the publication and preprint. This information indicates that the Candidate served as the leader author and was actively involved in the majority of research phases, co-designed the experiments, and played a significant role in the preparation of the manuscripts. The Candidate has clearly formulated research objectives and chose appropriate and various research methods that could help to answer for main questions. Msc Pranav Unnikrishnan presented research encompasses both field studies, which involved the analysis of climatic and soil data, as well as time-intensive laboratory experiments (experimental evolution and assessment of life history traits), alongside genetic analyses. This demonstrates the broad range of methodologies employed by Candidate and the diverse skill set acquired throughout the PhD project. The Candidate independently analysed the majority of the data using various statistical approaches and correctly interpreted the results, also identifying the limitations of the research conducted. In the final part of the dissertation, the Author proposed several potential research directions that could provide a more definitive explanation for the persistence of balanced genetic polymorphism in *6Pgdh* genes in bulb mites. In my opinion, all the above-mentioned arguments, demonstrate the ability of MSc Pranav Unnikrishnan to perform scientific work independently.

**4. Originality of the dissertation**

The central research question addressed in the doctoral dissertation pertains to one of the fundamental issues in evolutionary biology—namely, the maintenance of genetic variation in natural populations. Although this topic has been investigated extensively, with many studies emphasizing environmental heterogeneity as a key factor shaping genetic diversity, the precise mechanisms by which abiotic and biotic factors interact to sustain genetic polymorphism remain unclear. By employing bulb mites as a model organism, the Candidate was able to apply experimental evolution methods to test whether and how temperature and the intensity of sexual selection may jointly contribute to the

maintenance of genetic variation. One of the most significant findings presented in the dissertation is the interaction between temperature and social conditions (i.e., sexual selection) in maintaining genetic polymorphism in genes *6Pgdh* related to metabolism. I also found interesting the results showing no effect of temperature on key life history traits—such as development time and reproductive success—in males with different genotypes, suggesting the presence of highly complex mechanisms and interplay among various factors that underlie genetic polymorphism. I consider this to be an original solution to the scientific problem and important conclusion indicating new directions of research.

**5. Questions and/or criticisms to which the Reviewer expects the candidate to respond during the defence**

I would like to pose the following questions to the PhD Candidate:

1. In the discussion of the results, the potential role of females in shaping genetic polymorphism was not addressed. For instance, the possibility that females may selectively utilize sperm for fertilization—potentially in a context-dependent manner—could be taken under consideration. Although the summary briefly mentions sperm competition, it remains unclear whether females exert post-copulatory choice over which sperm fertilizes their eggs? What is Candidate opinion about such possibility?

Also, the study by Konior et al. (2006), cited in the introduction, reports reduced fertility in females that mated with SS males. Therefore, I would like to ask Candidate his opinion whether it would be valuable to explore whether female life-history traits can vary across genotypes depending on environmental or social conditions and by turn also influence genetic polymorphism in bulb mites?

2. There is a lack of information regarding the dispersal capabilities of bulb mites—for example, how and over what distances individuals are able to move. Yet, Chapter 1 discusses the role of gene flow, which implies some level of dispersal. I would like to ask Candidate to discuss this aspect. Specifically, could the data collected from multiple locations within a single city (e.g., Lublin or Kraków) be used to support or refute the potential role of gene flow in maintaining genetic polymorphism?"

3. What was the temperature differences among various populations collected in different places in Poland? E.g. the highest mean temperature difference? It is interesting in the context of temperatures used in experimental evolution. Was it similar range? How the results of these two studies can be compared in respect to temperature effect?

4. Bulb mites collected from natural populations (Chapter 1) were coming from: "different plant species collected from private gardens and botanical gardens". Thus, how many plant species altogether were collected and is it possible that their chemistry can affect *6Pgdh* polymorphism similarly to soil properties? Why data were collected in gardens and not in natural populations, as gardens are managed in a specific way, including soil fertilization and as mentioned by Candidate they can have specific microclimatic conditions.

5. In Chapter 2, in Material and method section it should be more clearly stated how many generations were taken into account and from each generation the allele frequencies were analysed (such information are shown in Fig. 8 but is should be mentioned in MM). I also wonder how much did it take in time and was it the same time for 12° C and 18° C?
It can be also explained how F-allele frequency was assesses in the source population – how many individuals were taken for analysis.

6. This is more general question: in the introduction it is written that "by the late 20th century, studies began to incorporate multiple alleles" whereas in the study of bulb mites only two allele gene is considered. I wonder which system would like to study Candidate in the future and what can be advantages and disadvantages of each of them?

Other minor comments, not necessary to discuss during PhD defence:
1. In introduction it is written: "circular hydrocarbons". I think it should be "cuticular hydrocarbons".
2. In Table 1, the last column could be split into two columns and significant p values could be marked differently to make it more easy to understand.
3. In the discussion of Chapter 2 various abbreviations for effective population size is used. It should be unified.
4. I also have a small formal comment: there is no Polish summary of the thesis, which according to requirements should be included into PhD thesis written in English.

I, hereby, declare that the reviewed PhD thesis by **Pranav Unnikrishnan** meets the criteria pursuant to art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended) and request that the Research Discipline Council of Biological Sciences of the Jagiellonian University in Kraków accepts **Pranav Unnikrishnan** for further stages of doctoral proceedings in the field of exact and biological sciences, in the discipline of biological sciences

YES/~~NO~~

I, hereby, request that the thesis is accepted with distinctions

~~YES~~/NO

Podpisane elektronicznie
przez Magdalena Maria Witek
(Certyfikat kwalifikowany) w
dniu 2025-08-18.

16.08.2025

Date

Reviewer's signature