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### **Review of doctoral dissertation**

**entitled: “A Journey Towards Identification of Paternal-Effect Genes and Exploration of Their Roles During Early Life Stages in Eurasian Perch, *Perca fluviatilis*”**  
**written by Abhipsa Panda**

### **General description of the doctoral dissertation**

The doctoral dissertation, submitted for evaluation, has been prepared at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn in Team of Reproduction and Development in Fish under the supervision of Dr hab. Daniel Źarski and Dr Sylwia Judycka. The work, written in English, includes 212 pages and consists of twelve chapters: 1) *General Introduction*, 2) *Research aims and hypotheses*, 3) *Paternal-effects genes revealed through sperm cryopreservation in Eurasian perch, *Perca fluviatilis**, 4) *Post-thaw storage of semen as tool towards revealing paternal-effect genes in Eurasian perch, *Perca fluviatilis**, 5) *Dynamic interplay of maternal and paternal contributions to offspring phenotype in Eurasian perch, *Perca fluviatilis**, 6) *General discussion*, 7) *Conclusion*, 8) *Implications and recommendation*, 9) *Literature*, 10) *Supplementary files*, 11) *Authors statements*, 12) *Published manuscript*. In addition, the list of frequently used abbreviations, and statements of co-authors concerning the participation in the preparation of publications composing the doctoral thesis have been included.

Ms. **Abhipsa Panda** is the first author of two publications: an original paper published in the *Scientific Reports* (2024, IF=3.9; 140 points of MNiSW) and a paper submitted to the BMC Genomics. In the third manuscript, which is under review in BMC Biology is a Co-author with equally contribution with the first Author - R. Debernardis. Based on the attached statements of co-authors regarding their involvement in the above publications, there is no doubt that MS Panda is a leading Author of them.

## **The analysis of the doctoral dissertation**

Overall, the thesis is well written and contains a clearly stated aims section, with three hypotheses corresponding to the three separate studies. These studies employed controlled maternal inputs and standardized fertilization procedures to isolate parental factors. The research undertaken by Ms. **Abhipsa Panda** falls within the scientific domain of the supervisor, focusing on mechanisms controlling fish reproduction, gamete quality, and factors influencing early development.

The manuscript begins with a General Introduction (18 pages) that presents the background and motivation for the research, framed within the context of “modern inheritance,” particularly non-genetic inheritance (NGI) and, more specifically, parental and paternal contributions to NGI in fish. The Author focused on identifying and characterizing paternal-effect genes (PEGs) in Eurasian perch, a model species for percid fishes, using a novel outcome-based approach. Rather than tracking specific sperm-derived molecules, the Candidate applied a reverse-inference strategy by manipulating sperm through cryopreservation, post-thaw storage stress, and comparisons between wild and domesticated broodstock. The resulting effects were then assessed through larval performance traits (such as: hatching rates, deformity rates, growth parameters, swim bladder inflation, and mortality during development) as well as whole-body transcriptomic analyses conducted just after hatching (0 days post-hatch), at the mouth-opening stage. This approach captures the functional impact of paternal inputs as expressed in the offspring, rather than inferring such effects solely from sperm content. The three complementary experimental designs are described in detail in Chapters 3, 4, and 5.

In the section relating to the paper entitled “*Paternal-effect genes revealed through sperm cryopreservation in Eurasian perch, *Perca fluviatilis*,*” the Author used milt cryopreservation as a targeted challenge test for sperm cells. Cryopreservation is a widely used method in aquaculture for sperm storage, synchronization of reproduction, and genetic resource management; however, its application also imposes strong selection pressure on sperm cells. Two experimental groups were established: eggs fertilized with fresh sperm (Fresh group) and eggs fertilized with cryopreserved sperm (Cryo group), with both sperm types obtained from the same males. Zootechnical performance parameters and transcriptomic profiles of the resulting offspring were evaluated at the larval stage. Most phenotypic traits were similar between the Fresh and Cryo groups; however, larvae from the Cryo group exhibited a significantly higher final weight at 15 days post-hatch.

Furthermore, transcriptomic analyses of newly hatched larvae revealed differential expression of genes associated with visual function and structural development, which were

identified as paternal-effect genes (PEGs). Particular emphasis should be placed on these findings, as they reveal, for the first time in fish, a significant influence of the paternal genome on visual system development. Specifically, *pde6g*, *opn1lw1*, and *rbp4l* were identified as novel PEGs. Importantly, the expression of these genes was not detected in unfertilized eggs, indicating that their expression is under paternal control.

In Chapter 4, “Post-thaw storage of semen as a tool towards revealing paternal-effect genes in Eurasian perch, *Perca fluviatilis*,” Ms. **Abhipsa Panda** used a 30-minute post-thaw storage period prior to fertilization as a precision stressor to investigate whether cryo-selected spermatozoa carry distinct molecular cargo with implications for offspring development. As expected, this treatment resulted in a sharp decline in sperm motility; however, it did not reduce fertilization success. This provided an opportunity to test whether sublethal deterioration of sperm function would affect larval performance and gene expression.

The Author demonstrated that post-thaw storage increased larval mortality compared with larvae obtained from freshly thawed sperm, while other evaluated zootechnical parameters remained unaffected. Furthermore, differential expression of *mfap4*, *gimap4*, *hlag*, and *pigr* was observed across larval stages. In contrast, the expression of other candidate paternal-effect genes, such as *pde6g* and *neol*, did not differ at later larval stages, likely due to compensatory mechanisms. Notably, downregulation of *pde6g* was detected only at the mouth-opening stage (also observed in the Fresh–Cryo study), suggesting a robust paternal imprint on sensory development.

In summary, the results show that even short-term post-thaw storage of cryopreserved sperm can induce significant changes in offspring gene expression and phenotype. Moreover, the magnitude and pattern of these responses appeared to be female-dependent. These findings indicate that, in Eurasian perch, maternal identity may modulate how post-thaw storage influences non-genetic inheritance factors carried by sperm

In Chapter 5, “*Dynamic interplay of maternal and paternal contributions to offspring phenotype in Eurasian perch, Perca fluviatilis*,” the Candidate exploited differences between wild-caught and domesticated males to investigate paternal effects operating in both natural and aquaculture-relevant contexts. This third experiment revealed that the maternal cytoplasm plays a key regulatory role in modulating paternal effects. Even under standardized female conditions, variation in embryonic and larval outcomes demonstrated that maternal factors modify the stability and interpretation of sperm-derived signals, highlighting an underappreciated maternal “gatekeeping” role.

Taking the obtained results into account, the Author suggests that progeny phenotype is shaped through a dynamic and stage-dependent interplay between maternal and paternal contributions. While maternal inputs dominate early development, likely due to preloaded

transcripts and egg provisioning, paternal influences emerge later and appear to exert a modulatory role in gene expression and phenotype refinement. Collectively, the three experiments presented by Ms. **Abhipsa Panda** provide a cohesive and complementary picture of paternal influences in fish development. It should be emphasized that conducting these studies at a high scientific level required the Candidate to employ a wide range of experimental procedures and research techniques, as well as to perform an extensive amount of laboratory work. It should also be underlined that Ms. Panda has demonstrated an extensive knowledge of the relevant scientific literature, reflected most notably in the highly insightful General Discussion (Chapter 6). It should be clearly emphasized that the studies on which the Candidate focused are highly significant in advancing our understanding of the nature of inheritance, developmental regulation, and the emerging role of paternal factors in shaping offspring phenotypes.

As a reviewer, I must note some minor editorial issues, such as incorrect chapter numbering on pages 9–12 and page 31. In addition, I would like to raise the following questions in response to “*A Journey Towards Identification of PEGs ...*”:

- What is your opinion on selective breeding strategies—specifically, should the selection of mothers be prioritized over fathers, given their greater influence on offspring development?
- With regard to the possible long-term effects of sperm cryopreservation, there are still insufficient data available on its potential impact on future offspring. How do you assess this limitation in the context of your findings

### **Conclusion**

In summary, the PhD thesis submitted for evaluation and written by Ms. Abhipsa Panda contains a substantial body of valuable results that significantly expand our knowledge of paternal-effect genes and non-genetic paternal effects in fish. The work also lays a strong foundation for novel applications in aquaculture, such as precision broodstock selection and molecular diagnostics for sperm quality. As such, the thesis represents a significant contribution to the emerging field of paternal epigenetics and adds an important dimension to our understanding of inheritance in vertebrates.

The doctoral dissertation of **Ms. Abhipsa Panda** meets all the required criteria, as it:

- demonstrates comprehensive theoretical knowledge expected of a PhD candidate in the discipline of animal science and fisheries;

- presents an original solution to a scientific problem, namely the identification and characterization of parental-effect genes in *Perca fluviatilis*;
- confirms the candidate's ability to conduct independent scientific research, as evidenced by the presented publications in which she is the first Author (one published in *Scientific Reports* and a second submitted to *BMC Genomics*) as well as a Co-author (manuscript under review in *BMC Biology*).

Summing up, I can state that the reviewed doctoral dissertation by **Ms. Abhipsa Panda** fulfils the requirements of The Act of July 20, 2018, Higher Education Law and Science (Journal of Laws of 2024, item 1571, with later amendments). The comprehensive experimental program conducted by the Candidate and the diversity of the research tools significantly expanded the Candidate's research competencies, enriching Her research skills.

Ms. Abhipsa Panda, the Author of the doctoral dissertation entitled "*A Journey Towards Identification of Paternal-Effect Genes and Exploration of Their Roles During Early Life Stages in Eurasian Perch, Perca fluviatilis*," submitted to the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn, has demonstrated the ability to conduct independent scientific research. The presented dissertation constitutes an original solution to a scientific problem, achieved through the candidate's solid theoretical knowledge in the discipline of animal science and fisheries.

Taking the above into account, the dissertation fully meets the requirements of the Act. Therefore, I apply for the admission of the Candidate to the public defense. Additionally, I recommend the doctoral dissertation of Ms. **Abhipsa Panda** for distinction (Attachment 1)

Respectfully

Magdalena Socha

Attachemnts:

1. Application for distinction for a doctoral dissertation entitled: "*A Journey Towards Identification of Paternal-Effect Genes and Exploration of Their Roles During Early Life Stages in Eurasian Perch, Perca fluviatilis*" written by Abhipsa Panda