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Vanessa Robles Rodríguez PhD / Full Professor

Department of Molecular Biology, University of León, Spain

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

1. Brief Summary of the Thesis

The dissertation investigates paternal-effect genes (PEGs) in the Eurasian perch, a topic traditionally underexplored in vertebrate developmental biology. The candidate applies an innovative outcome-based methodology that evaluates functional paternal contributions through larval performance and transcriptomic signatures rather than characterizing spermatozoal components. Three complementary experimental designs, comparison of fresh versus cryopreserved sperm, analysis of post-thaw sperm storage, and assessment of domesticated versus wild males, serve to elicit paternal perturbations and trace their consequences in larval phenotypes and gene expression. The research identifies several candidate PEGs related to visual, immune, and neurodevelopmental pathways. Together, these findings provide novel insights into the role of paternal contributions in early vertebrate development.

2. Assessment of Theoretical Knowledge

The dissertation clearly demonstrates that the candidate possesses broad and deep theoretical knowledge in the fields of fish reproductive biology, cryobiology, and transcriptomics.

The author integrates classical concepts of paternal inheritance with contemporary molecular and epigenetic theories, demonstrating familiarity with both historical and cutting-edge literature. The candidate shows an advance understanding of gene expression, and the mechanisms through which sperm-derived regulatory factors influence embryogenesis. The text illustrates theoretical maturity and the ability to contextualize results beyond applied aquaculture.



The introduction and the general discussion are also notably well crafted: they are clear, easy to follow, and supported by an updated and relevant bibliography. This careful framing strengthens the overall narrative of the dissertation and effectively situates the study within the current state of knowledge. Across chapters, the doctoral candidate accurately interprets data and provides hypotheses that show a good level of theoretical comprehension that is fully adequate for a PhD degree.

Conclusion: The dissertation meets the standards for theoretical knowledge and demonstrates a good understanding of the discipline.

3. Assessment of Ability to Conduct Independent Research

Based on the Author Contributions Section of the scientific articles included in this dissertation, it is clear that the candidate has been actively involved not only in the execution of the experiments but also in the conceptualization of the research. The experimental designs presented throughout the thesis are methodologically rigorous and well-founded, and they also demonstrate originality and thoughtful planning, reflecting a high level of critical thinking and problem-solving. Therefore, I conclude that the candidate has acquired the competence to conduct independent research and is capable of designing, implementing, and interpreting complex studies with rigor and creativity.

Conclusion: The work convincingly demonstrates the candidate's ability to independently plan, conduct, analyze, and interpret advanced scientific research.

4. Assessment of Originality

The dissertation takes an original approach by assessing paternal effects through “sperm manipulation” and offspring evaluation, rather than through molecular characterization of sperm. This practical, offspring-focused approach is both innovative and timely: by revealing consistent patterns indicative of paternal influence, the work addresses a long-standing gap in reproductive biology, where maternal effects have been extensively characterised while paternal contributions remain comparatively understudied. This conceptual shift introduces clear novelty to the thesis and situates it within an emerging line of research that is gaining increasing relevance.



Beyond this conceptual contribution, the dissertation also provides several concrete scientific advances that are broadly aligned with independent studies, which report comparable biological alterations, thereby reinforcing the robustness of its conclusions. As an example, the dissertation's conclusion regarding paternal influences on visual system development is strongly supported by emerging independent evidence. For instance, our study “miR-29a Is Downregulated in Progenies Derived from Chronically Stressed Males” reported altered developmental gene expression in offspring of stressed fathers, including the dysregulation of targets of miR-29a involved in eye formation. This convergence of findings reinforces the plausibility and significance of the candidate's results, further strengthening the impact of the dissertation. Interestingly we have also observed that direct early-life stress in the larvae produces ocular alterations not only at the molecular level but also at histological and anatomical levels. These observations collectively suggest that the eye may be especially sensitive to stress during early development, either as a direct target of early-life stressors or through paternal factors transmitted via the sperm that modulate developmental pathways.

I would particularly like to highlight the candidate's assertion regarding the impact of paternal contributions on progeny on page 91 of the dissertation, where it is stated that even when there are slight variations in sperm quality, can have clear effects on the progeny. This is also supported by independent evidence from our group, where we observed that progeny derived from males with reduced sperm quality exhibit molecular changes, including altered expression of key developmental genes and miRNAs, as reported in “Effect of low sperm quality on progeny: a study on zebrafish as model species” published by Riesco *et al.* in Scientific Reports. These findings reinforce, once again, the relevance and biological significance of the observations presented in the dissertation.

Conclusion: Overall, the dissertation demonstrates a thoughtful and original approach to studying paternal effects, combining practical sperm manipulations with careful evaluation of offspring outcomes. By revealing consistent patterns aligned with broader biological findings, the work not only fills a notable gap in reproductive biology but also positions the candidate's research within a timely and emerging area of genuine scientific interest.



5. Additional Remarks

All comments below are minor and intended as constructive suggestions:

The candidate suggested, appropriately framed as a hypothesis, that the observed overexpression of crystallins could indicate improved visual capacity and thus enhanced prey-capture ability. This is an interesting idea, and it is positive that it is presented cautiously. At the same time, it is important to note that molecular alterations, even when manifesting as overexpression, do not necessarily imply positive functional effects, just as downregulation can have negative consequences. Therefore, while the hypothesis is worth exploring, functional validation would be required to link molecular changes to actual visual performance.

A clear demonstration of the care with which this thesis has been written as well as of the broad knowledge of the candidate and her supervisors, is that the “Future Directions” section carefully addresses potential next steps. A deeper exploration of epigenetic mechanisms (DNA methylation, small RNAs, chromatin factors) could undoubtedly further strengthen mechanistic explanations. From my point of view, studies focused on miRNAs and also lncRNAs, represent particularly promising avenues that may yield interesting and insightful results in the future.

6. Final Recommendation

Taking into account all considerations presented above, I issue a positive and unequivocal review of the doctoral dissertation of Abhipsa Panda.

The dissertation meets all requirements for a PhD thesis in the relevant discipline. It demonstrates strong theoretical knowledge, clear ability to conduct independent scientific work, and delivers original findings of scientific and applied significance.

Therefore, I recommend that the Scientific Council accept the dissertation and, upon completion of the necessary formalities, award Abhipsa Panda the degree of Doctor in the appropriate discipline.

Vanesa Robles Rodríguez, PhD
Full Professor in Cell Biology
Department of Molecular Biology
University of León Spain

