Review of the Dissertation: ***Medaka as an Alternative Model Organism in the Study of Transcriptional Regulation***

The dissertation thesis by Ing. Simona Mršťáková is 121 pages long and features 61 Figures (impressive 41 of them in the Results section). The work focuses on establishing the Asian medaka fish as a novel model organism in developmental biology and applies it to study several phenomena, such as eye development in relation to the *Pax6* gene. The author presents one first-author publication, one collaborative paper, and one manuscript in preparation, indicating that the majority of the material in this dissertation has already undergone (presumably very thorough) peer review in recognized scientific journals. This high quality is reflected throughout the dissertation, which I consider, without any doubt, outstanding.

From a scientific aspect, the work contains enough information and evidence, showing the author's maturity to tackle scientific problems and be able to critically think. However, I believe the inclusion of more recent references would have been more than beneficial. The majority of references are quite old. While I understand that foundational discoveries may date back to, for example, the early 2000s, a quick search on PubMed for terms like *Pax6* and *eye* reveals dozens of papers published since 2015. Incorporating insights from these could have enriched the introduction, discussion and the overall scientific aspect of the work.

The language of the dissertation is flawless. According to the author's claim, grammar improvements were made using ChatGPT, which I find alright. Nevertheless, it has resulted in an excellent, albeit slightly sterile, text. Indeed, it would have been interesting to see at least the introduction or conclusion written in the author's own words to get a sense of their personal writing style. Otherwise, I have no complaints to the language aspect.

Regarding formatting, I was pleasantly surprised by the high quality of Figures in the Results section. The captions were clear (one exception below) and contributed significantly to the overall quality, making it easy to identify key differences, such as phenotypic variations. That said, I would recommend adopting a purple-green color scheme where possible, as it has become a standard in recent years, instead of the green-red scheme used in, for instance, Figure 40.

Suggestions for improvement:

1. In Figure 41, the number of cells and replicates should be specified, and statistical testing should be performed to draw more robust conclusions.
2. I found the term *Pax6.1 mutant* slightly confusing. Sometimes it seems to refer to both Pax6.1 HET and Pax6.1 KO mutants, while at other times, it is used in the singular form for HET mutant. For example, in Figure 40, the legend states that gene expression in the Pax6 mutant (singular/plural?) remain unchanged. However, for the markers *Nrl* and *Crx* in the Pax6.1 KO mutant, I do observe profound changes in expression of both. Please clarify this, as it might simply be a misunderstanding.

Discussion questions:

1. The dissertation dedicates considerable space to advocating for medaka as a new model organism (e.g., pages 96 and 118), which is understandable. However, could the author elaborate on the disadvantages of this model? What should researchers be cautious about when working with medaka? Or, to provoke further thought, in what ways is medaka inferior to zebrafish?

2. Figure 22 and the accompanying text highlight the involvement of *Meis* in eye development. As someone familiar with the WNT field, I am curious: Do you expect that the WNT-MEIS axis operates similarly in medaka eye development, as shown in mouse brain development (doi: 10.1242/dev.192054)? Some thought about involving of canonical vs. non-canonical WNT signaling?
3. How do you explain the presence of all three Pax6 genes (*Pax6.1, Pax6.2, Pax6.3*) in medaka, compared to only *Pax6.1* and *Pax6.2* in zebrafish? Was *Pax6.3* redundant for eye development in zebrafish then and that is why it is not present?
4. Could additional regulatory elements within the *Pitx2* gene or other signaling pathways contribute to the asymmetric expression of *Pitx2* in the epithalamus? How might these factors interact to fine-tune brain patterning during development?

Final evaluation:

Overall, I have no doubt that the author has demonstrated the ability to conduct independent scientific research and meets the requirements for the academic degree of PhD.

I therefore recommend the dissertation for defense and assign it the highest grade.

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