

An Optimized *In Situ* Hybridization Protocol to Study Early Developmental Patterns and Signaling Pathways in Paradise Fish (*Macropodus opercularis*)

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Supplementary Information

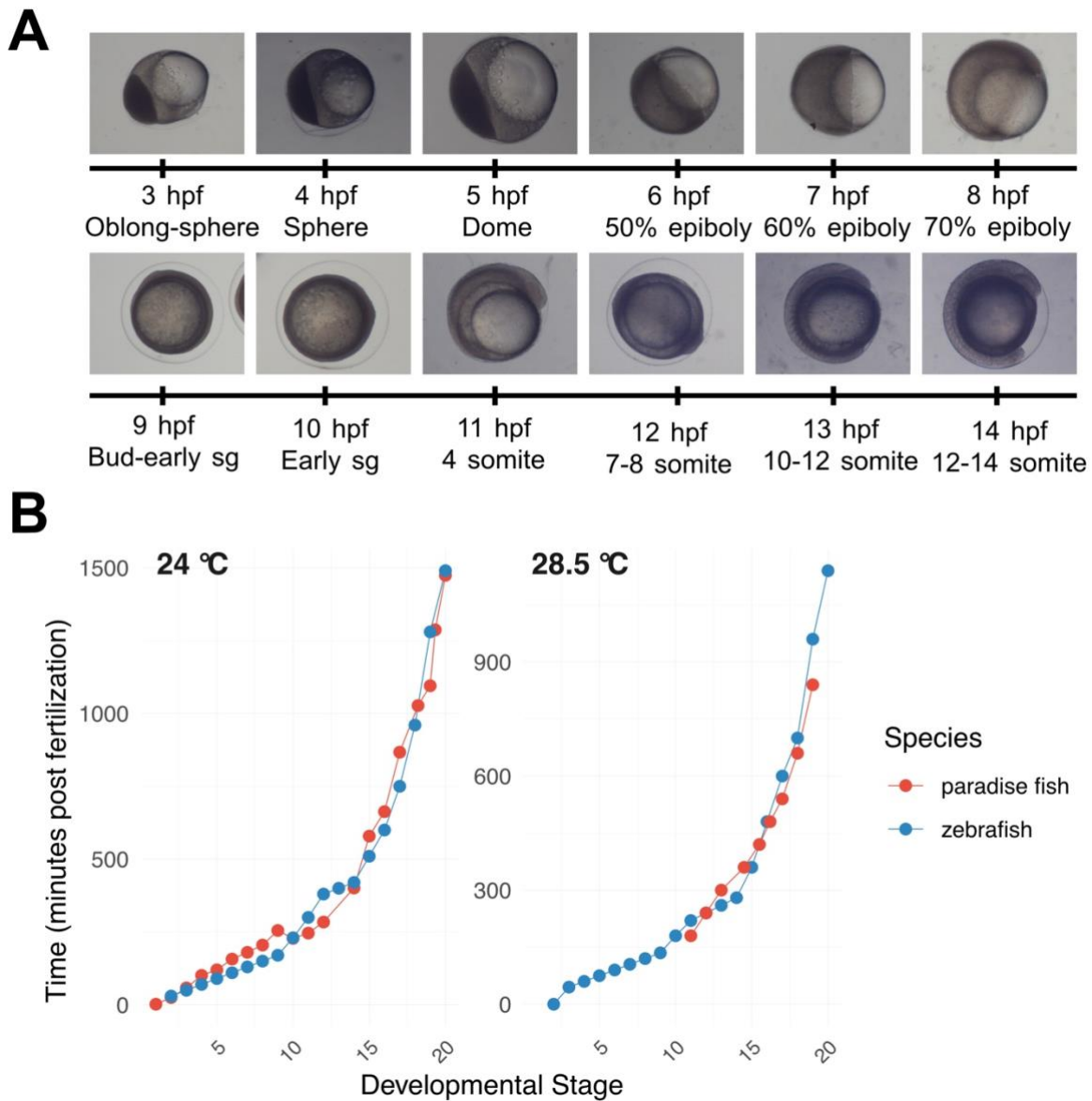


Figure S1: Standard development of paradise fish embryos at 28.5°C. (A) A developmental series showing the hourly procession of paradise fish development from blastula stage to the middle of somitogenesis. (B) Comparison of zebrafish and paradise fish developmental tempos both at room temperature (~24°C) and at 28.5°C. Based on (Kimmel et al. 1995; Yu and Guo 2018; Urushibata et al. 2021)

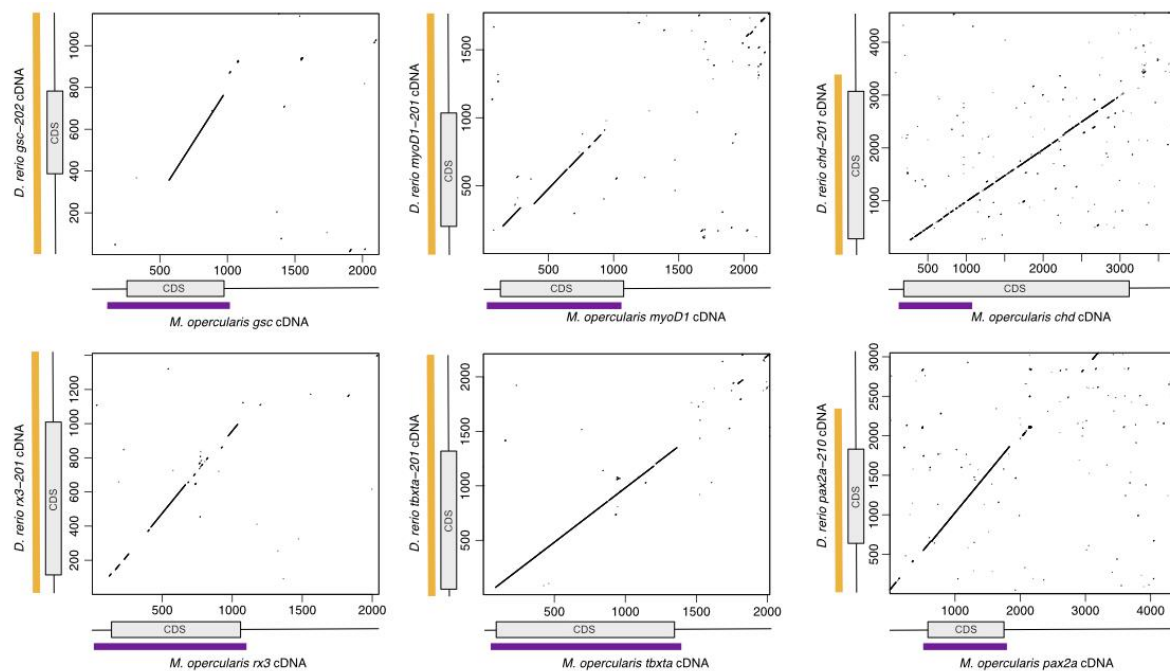


Figure S2: Similarity of zebrafish and paradise fish *in situ* probes used in this study. The complete CDS sequences of the paradise fish and zebrafish orthologs have been aligned using BLAST and plotted on a dot plot. The sequence parts used to create the *in vitro* anti-sense probes are highlighted with purple for paradise fish and yellow for zebrafish.

Supplementary references

- Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF (1995) Stages of embryonic development of the zebrafish. *Dev Dyn* 203(3):253–310.
- Urushibata H, Sasaki K, Takahashi E, Hanada T, Fujimoto T, Arai K, Yamaha E (2021) Control of Developmental Speed in Zebrafish Embryos Using Different Incubation Temperatures. *Zebrafish* 18(5):316–325. <https://doi.org/10.1089/zeb.2021.0022>
- Yu T, Guo Y (2018) Early Normal Development of the Paradise Fish *Macropodus opercularis*. *Russ J Dev Biol* 49(4):240–244. <https://doi.org/10.1134/s1062360418040057>