

**EVOLUTION OF THE SEX DETERMINATION PATHWAY IN THE GENUS *Oryzias*: A COMPARATIVE TRANSCRIPTOME ANALYSIS**

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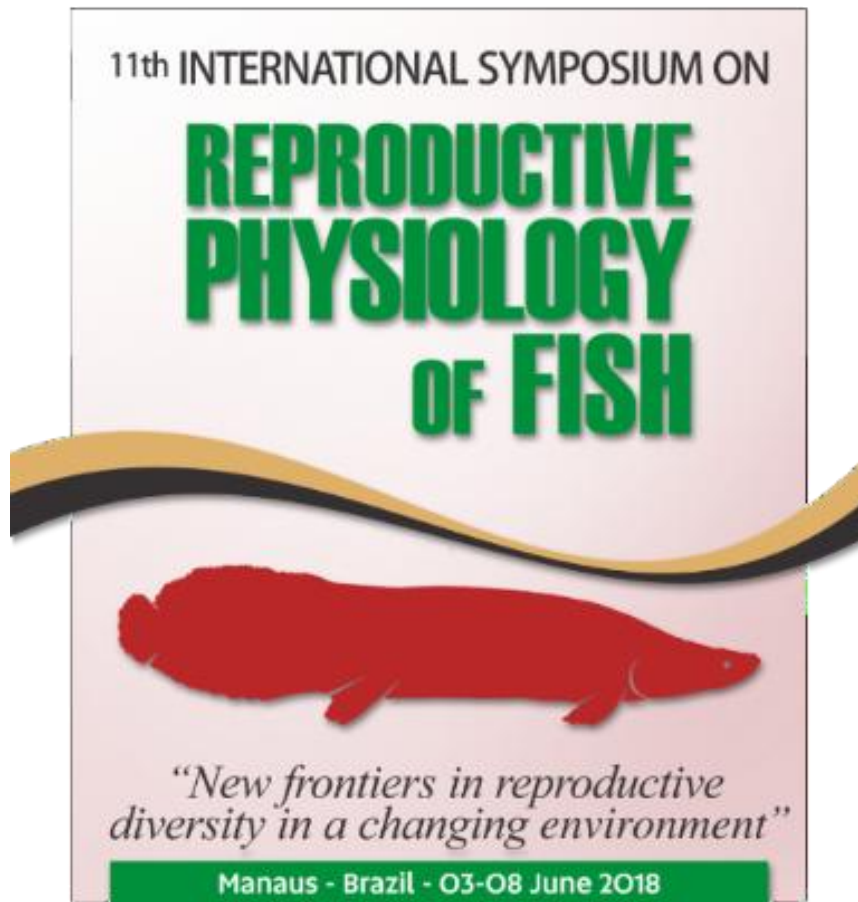
**Introduction :**In vertebrates, fish are the group with the highest diversity of sex determining genes. This variability is observed not only in species from distant branches of the phylogeny, but also within the same genus. The genus *Oryzias* is a well studied paradigm for this phenomenon. To understand how the sex determination genetic network evolved in closely related species, we analysed the genome and the transcriptome of adult gonads of four species of *Oryzias*: (1) *O. latipes* (XX/XY system where the sex determining gene (SD) on the Y is *dmrt1bY*, (2) *O. curvinotus* (XX/XY system with the same SD gene *dmrt1bY* on a homologous Y), *O. luzonensis* (XX/XY system but with a different SD gene, *gsdfY*, on a different linkage group) and *O. javanicus* (SD gene still unknown, ZZ/ZW sex determination system).

**Methods :**Three gonad samples of each sex were analyzed by RNA-seq in all four species. All sequences were mapped to the *O. latipes* reference genome using the RNA-seq aligner STAR and differentially expressed genes between testis and ovary were detected using DESeq2 (Bioconductor/R) for each species. Functionally enriched pathways and GO categories were found using the functional annotation tool DAVID (<https://david.ncifcrf.gov/>).

**Results and Discussion :** Histology of the adult gonads in four species showed similar structures. A global transcript analysis revealed enriched categories for genes involved in metabolic pathways also across all four species. Histogram analysis for an overview of the transcriptome data (considering Log Fold Change (LFC)>(3), Base Mean>100) showed that *O. javanicus* ovaries and testis are more similar to each other and were separated from the other species. Genes involved in gonadal development showed unexpected divergence in expression pattern between species. Several genes that are preferentially expressed in males of one species are expressed in females in the other species. *O. javanicus* has more genes exclusive differentially expressed in females (e.g. *activinRII*) and males (e.g. *cyp46a1*, *ptger3*) than the other species. The *fsta* (*follistatin a*) gene, a well-characterized “female” gene is highly expressed in ovary of *O. javanicus*, while in *O. latipes* this gene is higher expressed in males. Even though *O. latipes* and *O. curvinotus* share the same SD gene, the expression pattern of several genes critically involved in gonadal development are different. Analysis between the genome of *O. latipes*, *O. curvinotus* and *O. luzonensis* showed a conserved synteny for *dmrt1*. *dmrt1* in *O. luzonensis* is higher expressed in females, while in *O. latipes* and *O. curvinotus* the expression pattern is similar but it is higher expressed in males. Interestingly, the transcriptome showed a LFC significantly higher for *dmrt1* in *O. javanicus* males compared with the other species.

**Conclusion:** The final outcome showed a similar morphology of the gonad, despite of having different sex determining genes and regulatory pathways. The WZ species *O. javanicus* exhibited considerable differences in gonad gene expression compared to the XY species *O. latipes*, *O. curvinotus* and *O. luzonensis*.

# PROGRAM AND ABSTRACTS



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