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ESTROGEN RECEPTORS ARE SEX-DIFFERENTIALLY EXPRESSED IN TAMBAQUI (*Colossoma macropomum*) DURING SEX DIFFERENTIATION

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Introduction

Endogenous estrogens play several important roles in the different developmental processes in fish, such as sex differentiation. Their molecular actions are mediated by the estrogen receptors (ERα and ERβ2), which are ligand activated nuclear transactivation factors that control gene expression in all vertebrates. Besides, there is also a subfamily of orphan nuclear receptors closely related to the ERs, namely estrogen related receptors (ERRs) that has been postulated to influence estrogen signaling by either synergizing or competing with ERs in an apparent ligand independent manner. Here we report, for the first time, the identification and initial characterization of ERs and ERRs transcriptional levels in tambaqui (*Colossoma macropomum*) during sexual differentiation.

Methods

The *de novo* transcriptome individual libraries from six headless juvenile tambaqui (3 putative males and 3 putative females) during sex differentiation (from 20 to 33 mm total length) were assembled using the Trinity pipeline. Among other genes differentially expressed between males and females, the estrogen receptors were analyzed and phylogenetically characterized (by full genes identification on the *C. macropomum* genome), and the Coding Sequence (CDS) was used to deduce the protein.

Results and Discussion

In sex differentiating *C. macropomum*, both ERβ isoforms (ERβ1 and ERβ2), as well as ERRβ were observed to be differentially expressed in males and females. ERβ1 was exclusively expressed in males (fold change ≥ 2; FDR ≤ 0.05), whereas the ERβ2 isoform was higher expressed in males than females (fold change ≥ 2; FDR ≤ 0.05). The ERβ type binds to estrogens and activates the expression of responsive genes containing estrogen response elements in an estrogen-dependent manner. Interestingly, females expressed significantly higher levels of ERRβ (fold change ≥ 2; FDR ≤ 0.05), which is close related to the ER family, but it lacks the ability to bind to the estrogen. However, ERRs and ERs share common transcriptional target genes, such as the *cyp19a*, whose product converts C19 (androgens) to C18 (estrogens) steroids such as 17β-estradiol, the most active natural ER ligand.

Conclusion

Altogether, these findings raise new consistent hypotheses about sex differentiation in *C. macropomum* to therefore guide further studies. The different transcriptional levels observed for ERs and ERRβ between males and females suggest that feminization in the species might not be fully (if so) dependent of estradiol, as commonly observed in other teleost species.