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# Characterization of foxl2, sox9, and wt1a genes and their expression pattern as putative targets for sex identification in *Astronotus ocellatus* (*Agassiz*, 1831)

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**Abstract:** Using molecular markers is an effective method for sex identification in fish. In this regard, autosomal genes related to sex determination and differentiation may be of great interest. This study aimed to evaluate the expression pattern of Foxl2, Sox9, and Wt1a as molecular markers for sex identification in Oscar (*Astronotus ocellatus*). Molecular characterization and gene expression profiles for forkhead box l2 (Foxl2), SRY-box 9 (Sox9), and Wilm's tumor suppressor gene (Wt1a) were performed in male and female gonads. After euthanasia, histological and molecular analyses were conducted using ovarian and testis fragments of adult *A. ocellatus*. Histology was used for sex confirmation. The isolation of partial cDNA sequences encoding Foxl2, Sox9, and Wt1a from *A. ocellatus* was obtained by PCR using primers designed from conserved regions of the corresponding coding-domain sequence in other species. Sequencing of *A. ocellatus* Foxl2, Sox9, and Wt1a cDNA fragments allowed us to perform RT-qPCR assays in male and female gonadal tissue to analyze the gene expression profile. RT-qPCR revealed markedly gender-influenced gene expression patterns for all target genes. Foxl2 mRNA levels were significantly higher in the ovary than in the testes. At the same time, Sox9 and Wt1a were upregulated considerably, and mRNA levels for Sox9 and Wt1a were 8- and 9-fold higher in the testis compared to the ovary. Our findings indicate that Foxl2 expression may serve as a molecular marker for the identification of males. At the same time, the genes Sox9 and Wt1a are effective in identifying females in *A. ocellatus* gonads up to 12 months of age.

**Keywords:** Wilm's tumor suppressor gene, Sex determination, Sex identification, Molecular markers.

## 1. Introduction

Sex identification in fish is a matter of great importance in aquaculture, especially in breeding in captivity. Through appropriate sexing, it is possible to establish a broodstock fish with an adequate proportion of males and females. Therefore, fingerling production can be improved by reducing costs from unappropriated breeder maintenance (Rodriguez-Hernández et al., 2025). However, sex identification in species that do not present sexual dimorphism and heteromorphic sex chromosomes is limited, making it necessary to use new sexing approaches.

Molecular markers are a highly efficient method for sexing fish (Zhang et al., 2017; Dai et al., 2021). Autosomal genes related to sex determination and differentiation have great potential as helpful sex markers. This is because these genes are differentially expressed in a sex-specific manner, playing a determining role in testicular and ovarian formation (Bertho et al., 2016).

In fish, sex determination and differentiation involve a complex network of molecular events that are influenced by various genetic and environmental mechanisms (Nagahama et al., 2021). Many studies have reported the involvement of autosomal genes in sex determination in fish, including the forkhead box l2 (Foxl2) gene (Bhat et al., 2016b), SRY-box 9 (Sox9) (Bhat et al., 2016a), and Wilm's tumor suppressor gene (Wt1a) (Chen et al., 2015). The Foxl2 gene is a family member of the forkhead transcription factors characterized by the conserved DNA-binding forkhead box domain (Tucker, 2022). Among the functions of Foxl2 in sex determination and differentiation, Foxl2 controls Cyp19a1 expression, which encodes the P450 aromatase. Consequently, Foxl2, by regulating this steroidogenic enzyme, has a strong influence on ovarian differentiation (Tucker, 2022; Liu et al., 2015). In addition, Foxl2 is also responsible for suppressing testicular differentiation by repressing Sox9 (Tucker, 2022).

Sox9 and Wt1a have been associated with testicular development and differentiation. Sox9 is a member of the E group of the SOX family in which genes have a DNA binding domain called HMG-box (Sreenivasan et al., 2022). This gene encodes the transcription factor Sox9, a crucial factor in gonadal differentiation in vertebrates (Gonen and Badge, 2019). Considering the role of Sox9 in fish testicular differentiation, its positive regulation of the Amh gene has been emphasized. Amh is responsible for Müller's ducts regression, which, in another instance, would differentiate to form the uterine tubes and uterus (Wagner, 2023). Thus, Sox9, through the regulation of Amh expression, is a key factor for testicular differentiation.

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