

MINGHUA ZHANG : DEVELOPMENT OF SEX-SPECIFIC DNA MARKERS IN
SNAKESKIN GOURAMI (*Trichopodus pectoralis*) AND THEIR APPLICATION FOR
IDENTIFICATION OF NEOMALES. THESIS ADVISOR: PROF. SURINTORN
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PRODUCTION

Sexual dimorphism in growth characteristics is observed in many economically important fish species. The snakeskin gourami (*Trichopodus pectoralis*), an economically important freshwater fish widely cultured in Southeast Asia, exhibits pronounced female-biased growth. Recently, estradiol-induced sex reversal technology has been established to produce all-female populations for commercial purposes. In addition, an indirect method to generate neomales could be an alternative strategy to produce all-female fish populations, and a genotypic sex-specific marker could offer an efficient procedure to produce neomales. However, information on sex-specific markers in snakeskin gourami remains limited. Therefore, this study aimed to develop sex-specific markers and apply them to determine the genotype of neomales.

In Experiment I, transcriptome-based SNP analysis using RNA sequencing of ovarian and testicular tissues identified 21 candidate sex-specific RNA SNPs. Validation by Sanger sequencing and PACE assays confirmed nine sex-specific SNPs identified from RNA-seq, that were heterozygous in males and homozygous in females. Among these SNPs, six SNPs including SNP_2, SNP_3, SNP_4, SNP_5, SNP_12, and SNP_15 were located in *ift172*, *man2a1*, *crk*, *ezh2*, *cs*, and *cast*, respectively. However, these SNPs were not detected in genomic DNA, suggesting that RNA editing may be involved in sex determination. Additionally, male-biased expression of *adar* suggested that RNA editing may contribute to sex differentiation in snakeskin gourami. The remaining three SNPs (SNP_19, SNP_20, and SNP_21) were located in *kap3l*. Subsequent validation at the genomic DNA level showed that SNP_19, SNP_20, and SNP_21 were concordant with nucleotide variation in genomic DNA, suggesting that these three SNPs could be

used for sex genotyping. Collectively, these findings demonstrate the identification of sex-specific SNP markers for sex determination in snakeskin gourami.

In Experiment II, neomale broodstock were generated through dietary administration of 17 α -methyltestosterone (MT), while the control group consisted of naturally developed broodstock. Genetic sex was determined using sex-specific SNP markers (SNP_19, SNP_20, and SNP_21) via Sanger sequencing and PACE SNP assays. All markers exhibited consistent genotyping patterns, with MT-treated males and control males showing heterozygous genotypes, whereas MT-derived neomales and females displayed homozygous genotypes. Progeny testing confirmed that crosses between neomales (XX) and females (XX) consistently produced 100% female offspring. These results support an XX/XY sex-determination system in snakeskin gourami. Therefore, sex-specific SNP markers could be applied to identify neomales.

In Experiment III, neofemales induced by 17 β -estradiol (E2) were genetically confirmed using SNP_19, SNP_20, and SNP_21. E2-treated neofemales and control males exhibited heterozygous genotypes, whereas E2-treated females displayed homozygous genotypes. Crosses between neofemales (XY) and males (XY) produced male-biased progeny (male: $73.34 \pm 3.16\%$), consistent with an XX/XY sex determination system. Therefore, sex-specific SNP markers could also be applied to identify neofemales.

In summary, this study developed reliable sex-specific SNP markers and demonstrated their application in genetic sex identification. The presence of heterozygous SNPs in males suggests an XX/XY sex-determination system in snakeskin gourami. These sex-specific SNP markers can be applied for genotyping not only neomales but also neofemales.

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