CHAPTER I
INTRODUCTION

1.1 Introduction

Sex determination in teleost fishes exhibits remarkable diversity and complexity compared with that in other vertebrates. Unlike mammals and birds, in which sex is typically governed by highly conserved sex chromosome systems, fishes display a wide variety of sex determination patterns that differ markedly among taxa (Devlin and Nagahama, 2002; Mank and Avise, 2009). These systems include environmental sex determination (ESD), in which sex differentiation can be influenced by external environmental conditions, resulting in variable sex ratios among populations. In contrast, under genetic sex determination (GSD), sexual fate is primarily determined by sex chromosomes or sex-linked genomic loci, including simple systems such as XX/XY and ZZ/ZW, as well as multiple sex chromosome systems (Devlin and Nagahama, 2002; Mank and Avise, 2009). In addition, mixed mechanisms have been reported in which genetic factors and environmental cues may jointly influence sexual development (Baroiller and D'Cotta, 2001; Baroiller et al., 2009). However, in many teleost fishes, sex chromosomes are often poorly differentiated or cytogenetically indistinguishable, which limits the applicability of morphological and classical cytogenetic approaches for genetic sex identification (Mank and Avise, 2009). Under such conditions, the development of genetic markers provides an effective solution for accurately determining genetic sex (Mei and Gui, 2015).

In teleost fishes, distinct heteromorphic chromosomes are rarely observed (Cioffi et al., 2017). Instead, genetic markers derived from sex-associated genomic regions provide an effective approach for genetic sex identification, enabling reliable discrimination at the DNA level across developmental stages. These sex-linked markers have been widely applied in studies of sex chromosome systems and aquaculture breeding programs, particularly for early sex identification and monosex population production (Liu and Cordes, 2004; Luckenbach et al., 2017; Mei and Gui, 2015; Lu et al., 2024).

A wide range of molecular marker systems has been developed for genetic analysis in fishes, including randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), and insertion–deletion polymorphisms (InDels) (Liu and Cordes, 2004; Mei and Gui, 2015). These markers have been successfully applied in population genetics and sex-linked marker development. However, limitations such as low reproducibility, dominant inheritance, or restricted genomic coverage may reduce their efficiency in large-scale applications. In recent years, single-nucleotide polymorphisms (SNPs) have emerged as the most widely used genetic markers in fish studies. Owing to their high abundance throughout the genome, high genetic stability, and compatibility with high-throughput genotyping platforms, SNP markers provide a reliable and cost-effective approach for genetic sex identification and breeding-related analyses (Mei and Gui, 2015; Zhao et al., 2019; Liu et al., 2018). Consequently, transcriptome-based SNP discovery has become an effective strategy for identifying sex-linked loci in non-model aquaculture species.

The snakeskin gourami (*Trichopodus pectoralis*) is an economically important freshwater fish widely cultured in Southeast Asia (Klinmalai et al., 2021; Panthum et al., 2021; Dinh-Hung et al., 2023). This species exhibits pronounced sexual size dimorphism, with females showing faster growth and larger body size than males, resulting in higher commercial value (Boonanuntanasarn et al., 2020; Kabpha et al., 2023). However, the sex determination mechanism of snakeskin gourami (*T. pectoralis*) remains poorly understood, and reliable methods for early genetic sex identification are currently lacking (Panthum et al., 2021; Jantawongsri et al., 2025). These limitations highlight the necessity for developing robust molecular markers to enable precise genetic sex identification in this species. The present study aimed to develop reliable sex-specific DNA markers in snakeskin gourami using transcriptome sequencing and SNP validation. By identifying and verifying sex-linked SNP loci, this study seeks to establish an effective molecular approach for genetic sex identification, thereby providing a scientific basis for broodstock management and the potential development of sustainable monosex breeding strategies.

1.2 Research objectives

1.2.1 To develop sex-specific DNA markers and validate them for identifying the genotypic sex of snakeskin gourami through transcriptome-based SNP discovery.

1.2.2 To apply the developed sex-specific markers to identify the genotypic sex of hormone-induced neomales or neofemales.

1.3 Research hypotheses

1.3.1 There are consistent and heritable differences at the nucleotide level (e.g., SNPs) between genetic males and females in *Trichopodus pectoralis*. Consequently, sex-linked SNPs can be developed into reliable DNA markers for identifying the genotypic sex in snakeskin gourami.

1.3.2 The sex determination system in snakeskin gourami is, at least in part, controlled by genetic factors. Therefore, the identified sex-specific DNA markers can be used to determine the genotypic sex of neomales (phenotypic males induced via hormone treatment) or neofemales (phenotypic females induced via hormone treatment).

1.4 Scope of the study

This study focuses on the development of sex-specific markers using transcriptome-based discovery at the transcriptional level. To ensure accurate sex determination, the collected gonads were divided into two parts: one portion was preserved at −80°C for total RNA extraction, while the other underwent histological examination to confirm phenotypic sex and developmental stage.

To develop reliable sex-specific markers, transcriptome information from testes and ovaries of juvenile and adult fish was used. Comparative analysis of male and female transcriptomes from both juvenile and adult stages enabled the identification of candidate sex-linked genetic markers associated with genotypic sex. Following bioinformatic filtering, selected candidate sex-specific RNA SNPs were validated in another fish population. In addition, these candidate RNA SNPs were validated in genomic DNA to confirm their potential as sex-specific markers.

PCR-based genotyping assays were developed to assess the presence and sex-

association of these markers in a broader population. Once validated, these molecular markers were applied to identify the genotypic sex of hormonally induced neomales (phenotypic males with a presumptive XX genotype). Neomales were crossed with normal females, and the resulting offspring were sexed to determine sex ratios. Similarly, the molecular markers were used to identify the genotypic sex of hormonally induced neofemales (phenotypic females with a presumptive XY genotype). Neofemales were crossed with normal males, and the resulting offspring were sexed to determine sex ratios. Overall, this study aims to establish a robust, non-invasive, and reliable method for sex identification, which could support the production of monosex populations in *T. pectoralis* aquaculture, thereby enhancing production efficiency and economic value.

1.5 Expected benefits

The development of SNP-based molecular markers would enable precise identification of the genetic sex of *T. pectoralis*. This technology would allow for early sex determination, particularly during developmental stages when fish have not yet exhibited sex-related morphological differences. Therefore, this study aims to establish a robust, non-invasive, and reliable method for sex identification, which could support the production of monosex populations in *T. pectoralis* aquaculture.

Since female *T. pectoralis* grow faster than males, all-female production is preferred in fish farming. Recently, 17-β estradiol-induced sex reversal has been implemented to produce all-female populations. To directly generate all-female populations without hormone use, neomales are required. Neomales can be produced through dietary supplementation with 17-α methyltestosterone, and progeny testing is typically used to identify their genotypic sex. However, this approach is time-consuming, labor-intensive, and requires large hatchery facilities.

The application of sex-specific markers would allow for rapid, non-labor-intensive, and facility-efficient genotypic sex determination, enabling more efficient production of neomale broodstock. These neomales can be selectively mated with normal females to produce all-female progeny, which typically exhibit faster growth and higher market value.

This hormone-free strategy offers a sustainable, consumer-friendly, and

environmentally safe alternative to traditional hormone-based sex reversal methods, thereby enhancing production efficiency in *T. pectoralis* aquaculture. Furthermore, this study contributes critical knowledge toward understanding the genetic basis of sex differentiation and supports the long-term objective of developing stable, monosex breeding programs. Overall, the findings from this study are expected to provide both practical applications for the aquaculture industry and scientific insights into the molecular mechanisms of sex determination in teleost fishes, laying a foundation for future genetic and reproductive studies in *T. pectoralis*.

1.6 References

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