

CONTENTS

	Page
ABSTRACT IN THAI.....	I
ABSTRACT IN ENGLISH.....	III
ACKNOWLEDGEMENT	V
CONTENTS	VII
LIST OF TABLES.....	XIII
LIST OF FIGURES.....	XV
LIST OF ABBREVIATIONS.....	XVII
CHAPTER	
I INTRODUCTION	1
1.1 Introduction.....	1
1.2 Research objectives.....	3
1.3 Research hypotheses	3
1.4 Scope of the study.....	3
1.5 Expected benefits.....	4
1.6 References.....	5
II LITERATURE REVIEWS.....	7
2.1 Biological characteristics of snakeskin gourami	7
2.2 Sex determination system in fish	9
2.3 Molecular Markers for Sex Determination in Teleost Fishes.....	11
2.4 Sex reversal and monosex breeding strategies in aquaculture.....	14
2.5 References.....	18
III Transcriptome-Based SNP Discovery Revealed SNPs Associated With Sex Determination and With RNA Editing Involved in Sex Differentiation In Snakeskin Gourami (<i>Trichopodus pectoralis</i>)	27
3.1 Abstract.....	27
3.2 Introduction.....	28

CONTENTS (Continued)

	Page
3.3 Materials and methods.....	30
3.3.1 Ethics statement.....	30
3.3.2 Experimental design, experimental fish, and sample collection	30
3.3.3 RNA sequencing, read pre-processing, <i>de novo</i> transcriptome assembly, and SNP Identification	30
3.3.4 Validation of candidate RNA SNPs and their corresponding DNA variants using Sanger method.....	32
3.3.4.1 Sample collection and histological study	32
3.3.4.2 Total RNA extraction, reverse transcription PCR, and Sequencing	32
3.3.4.3 Genomic DNA extraction, DNA variant analysis using Sanger sequencing.....	34
3.3.5 Validation of candidate RNA SNPs and their corresponding DNA variants using PACE SNP analysis.....	40
3.3.6 Validation of sex specific DNA SNPs in other fish populations.....	41
3.3.6.1 Sample collection and DNA variant analysis using Sanger sequencing	41
3.3.6.2 DNA variant genotyping using PACE SNP assay.....	42
3.3.6.3 DNA variant genotyping using T7 endonuclease I assay	42
3.4 Results.....	42
3.4.1 Sex-specific SNPs identified from gonadal transcriptomes of juvenile and adult <i>T. pectoralis</i>	42
3.4.2 Validation of candidate sex-specific RNA SNPs	43

CONTENTS (Continued)

	Page
3.4.3 Differential expression and transcript annotation of 9 sex-associated SNPs in juvenile and adult gonads of <i>T. pectoralis</i>	46
3.4.4 Validation of candidate sex-specific DNA SNPs in the genome using Sanger sequencing, PACE SNP analysis.....	48
3.4.5 Differential expression in <i>adar</i> might be associated with RNA editing in males	49
3.4.6 Validation of sex specific SNPs in SNP_19, SNP_20, and SNP_21 in other fish populations by Sanger sequencing and PACE assay	50
3.4.7 Identification of RNA SNPs of SNP_19, SNP_20, and SNP_21 and their DNA variants using T7EI	51
3.5 Discussion	53
3.6 Conclusion.....	59
3.7 Acknowledgements	59
3.8 References.....	60
IV Validation of XX/XY Sex Determination and All-Female Production Via 17α-Methyltestosterone-Induced Neomales in Snakeskin Gourami (<i>Trichopodus Pectoralis</i>).....	67
4.1 Abstract.....	67
4.2 Introduction.....	68
4.3 Materials and methods.....	72
4.3.1 Ethics statement.....	72
4.3.2 Fish preparation	72
4.3.3 Neomale broodstock production.....	72
4.3.4 Progeny testing.....	73
4.3.5 Histological examination of the gonads	74
4.3.6 Genotypic analysis using sex-specific SNP	74

CONTENTS (Continued)

	Page
4.3.7 Data analysis.....	75
4.4 Results.....	77
4.4.1 Genotypic identification of control males, MT-treated males, MT-derived neomales, and females broodstock by Sanger sequencing.....	77
4.4.2 Genotypic identification of control males, MT-treated males, MT-treated neomales, and females using PACE genotyping.....	80
4.4.3 Progeny testing of control males, MT-treated males, and MT-derived neomales using the squash method and histological analysis	80
4.4.4 Progeny testing of control males, MT-treated males, and MT-derived neomales using PACE genotyping	83
4.5 Discussion	86
4.5.1 MT-induced masculinization and molecular identification of neomales in snakeskin gourami.....	86
4.5.2 Neomale-based all-female production and reproductive performance of MT-treated broodstock.....	87
4.5.3 Progeny testing confirms the XX/XY sex determination system and the effectiveness of neomale-based all-female production.....	88
4.6 Conclusion.....	89
4.7 Acknowledgements	89
4.8 References.....	89
V Evidence for an XX/XY Sex Determination System in Snakeskin Gourami (<i>Trichopodus Pectoralis</i>) Revealed by 17β-Estradiol-Induced Neofemale.....	94

CONTENTS (Continued)

	Page
5.1 Abstract.....	94
5.2 Introduction.....	95
5.3 Materials and methods.....	99
5.3.1 Ethics statement.....	99
5.3.2 Fish breeding and larval production	99
5.3.3 Production of neofemales.....	100
5.3.4 Progeny testing of control group and E2-treated female group, and E2-treated neofemale group.....	101
5.3.5 Histological examination of the gonads	101
5.3.6 Genotypic analysis using sex-specific SNP	102
5.3.7 Production of supermale	103
5.3.8 Progeny testing of the control male group and supermale group	103
5.3.9 Histological analysis of gonads.....	104
5.3.10 Genotypic analysis using sex-specific SNP.....	104
5.3.11 Data analysis.....	106
5.4 Results.....	106
5.4.1 Genotypic identification of control females, E2-treated females, E2-treated neofemales, and males broodstock by Sanger sequencing.....	106
5.4.2 Progeny testing of control females, E2-treated females, and E2-treated neofemales using the squash method and histological analysis.....	110
5.4.3 Reproductive performance and genotypic identification of control males and supermales	113
5.4.4 Progeny testing of control males and supermale using the squash method and histological analysis.....	116
5.5 Discussion	120

CONTENTS (Continued)

	Page
5.5.1 Molecular validation of estrogen-induced sex reversal using sex-specific SNP	120
5.5.2 Functional validation of E2-induced neofemales through phenotypic and progeny sex ratio analyses	121
5.5.3 Validation of sex-specific SNP markers for genetic sex identification in broodstock	123
5.5.4 Functional confirmation of the XX/XY sex determination system through progeny testing	124
5.6 Conclusion.....	125
5.7 Acknowledgements	125
5.8 References	126
VI OVERALL CONCLUSION AND IMPLICATION.....	130
6.1 Overall conclusion.....	130
6.2 Implication.....	132
APPENDX.....	135
BIOGRAPHY.....	146