CHAPTER II

LITERATURE REVIEW

2.1 Biological Characteristics of Snakeskin Gourami

The snakeskin gourami (*Trichopodus pectoralis*) is a freshwater teleost belonging to the family Osphronemidae (Figure 2.1 and Table 2.1), which also includes other labyrinth fishes such as the giant gourami (*Osphronemus goramy*) and the three-spot gourami (*Trichopodus trichopterus*). *T. pectoralis* is one of the most economically important gourami species in Southeast Asia and is widely cultured for both food consumption and ornamental purposes (Nakharuthai et al., 2022). Its natural range extends across Thailand, Cambodia, Vietnam, Laos, Malaysia, and Indonesia, with introductions into Myanmar, Sri Lanka, and India to support aquaculture (Dinh-Hung et al., 2023; Klinmalai et al., 2021; Ramadhani et al., 2020; *The State of World Fisheries and Aquaculture 2020*, 2020; Vromant et al., 2001).

Figure 2.1 Snakeskin gourami (*Trichopodus pectoralis*).

Table 2.1 Taxonomy of the snakeskin gourami (*Trichopodus pectoralis*).

Rank	Classification
Phylum	Chordata
Class	Actinopterygii
Order	Anabantiformes
Family	Osphronemidae
Genus	*Trichopodus*
Species	*Trichopodus pectoralis*

Reference: Regan, 1909.

In Thailand, the snakeskin gourami has long been integrated into rice–fish farming systems, supported by its strong tolerance to hypoxic conditions and broad ecological adaptability (Ninwichian et al., 2018). The ecological success of *T. pectoralis* is largely attributed to the labyrinth organ, a suprabranchial structure that enables aerial respiration, allowing the species to thrive in oxygen-poor habitats such as swamps, floodplains, and rice paddies (Martin and Graham, 1998; Froese, 2006). Together with its omnivorous feeding habits, ranging from zooplankton and insect larvae to algae and detritus, these traits promote stable growth in both natural and cultured environments and make the species highly compatible with integrated rice–fish farming systems (Panthum et al., 2021).

Beyond its value as a food fish, *T. pectoralis* also holds cultural and economic importance in Thailand, where dried and salted gourami fillets are considered a traditional delicacy, and its robust physiology and distinctive body patterning support demand in the ornamental fish trade (Klinmalai et al., 2021). However, rapid urbanization and the reduction of agricultural land have constrained traditional extensive production systems, while increasing consumer demand, particularly in Thailand, where *T. pectoralis* consistently ranks among the top five farmed freshwater fishes, has driven a transition toward semi-intensive and intensive aquaculture practices (Boonanuntanasarn et al., 2020; Kabpha et al., 2023). Despite these developments, current production levels remain insufficient to satisfy both domestic consumption and export markets (Dinh-Hung et al., 2023).

Snakeskin gourami is a gonochoristic species exhibiting clear sexual size

dimorphism, in which females generally grow faster and attain a larger body size than males (Boonanuntanasarn et al., 2020; Kabpha et al., 2023). Under culture conditions, females have been reported to reach harvest size approximately 20–30% larger than males, making female-biased populations economically advantageous in aquaculture production (Boonanuntanasarn et al., 2020; Kabpha et al., 2023). This female-biased growth advantage highlights the potential value of an all-female culture in aquaculture profitability. Yet, the genetic and environmental mechanisms underpinning sex determination remain poorly understood. Like many teleosts, *T. pectoralis* may possess homomorphic sex chromosomes that cannot be distinguished cytogenetically, complicating efforts to manipulate sex for production purposes (Araya-Jaime et al., 2017; Artoni et al., 2015). These knowledge gaps have hindered selective breeding programs, underscoring the need for molecular approaches to address them. Recent advances in genomics and transcriptomics have begun to provide tools for sex identification and genetic improvement. Subsequent sections of this review will explore these developments in the broader context of fish aquaculture.

2.2 Sex determination system in fish

Sex determination mechanisms in teleost fishes are remarkably diverse, ranging from classical male-heterogametic (XY) and female-heterogametic (ZW) systems to more complex arrangements (Fang et al., 2025). These systems often differ not only among families but also among closely related species. The identification of these systems has relied on a variety of cytogenetic, molecular, and genomic approaches, including karyotyping, fluorescent in situ hybridization (FISH), microsatellite-based linkage mapping, high-density single-nucleotide polymorphisms (SNPs) genotyping, and high-throughput sequencing technologies (e.g., RAD-seq, GWAS, and whole-genome assembly) (Devlin and Nagahama, 2002). To provide an overview of the breadth of sex determination diversity and the tools used to study it, Table 2.2 summarizes representative teleost species, their documented sex determination systems, the methods used for their characterization, and corresponding key references. This comparative framework highlights the complexity of sex determination across fishes and underscores the need for integrative molecular tools to support monosex breeding and sex-control strategies in aquaculture.

Two broad categories are often distinguished: genotypic sex determination (GSD) and environmental sex determination (ESD). In GSD systems, sex is primarily defined by alleles at a sex-determining locus, and the classic heterogametic models XX/XY (male heterogamety) and ZZ/ZW (female heterogamety) are both widely represented in teleosts (Mank and Avise, 2009). However, more complex systems exist, including multiple sex chromosome arrangements such as X_1X_2Y, ZW_1W_2, or X0/XX, as reported in several cyprinids and flatfishes (Kitano and Peichel, 2012). In contrast, ESD occurs when abiotic or social cues determine phenotypic sex, regardless of the genotype. Temperature is the most influential factor, with temperature-dependent sex determination widely reported in both aquaculture and wild populations (Baroiller and D'Cotta, 2001; Devlin and Nagahama, 2002). Other environmental variables, such as pH, photoperiod, or density, have also been shown to bias sex ratios in some taxa (Reddon and Hurd, 2013). Importantly, mixed systems (GSD + ESD) occur in many species, where genetic cues establish a predisposition that can be overridden by environmental signals (Sandra and Norma, 2010).

Cytogenetic approaches, which visualize heteromorphic chromosomes through karyotyping or FISH, have proven effective in species with highly differentiated sex chromosomes, such as tilapia and medaka (Harvey et al., 2002; Matsuda et al., 1998). However, for most fish with homomorphic chromosomes, these methods provide little insight (Charlesworth and Mank, 2010). Breeding experiments, often involving sex-reversed individuals, can help reveal patterns of inheritance, but such approaches are labor-intensive, time-consuming, and limited to a handful of model or commercial species (Piferrer, 2001).

Molecular approaches are increasingly filling this gap by offering a powerful means to detect sex-linked loci in species lacking cytogenetic signals. Association mapping, linkage analysis, and high-throughput sequencing technologies have revealed small regions of suppressed recombination associated with sex in a wide range of fishes (Bao et al., 2019). These findings highlight that sex chromosomes in teleosts are often young and dynamic, with frequent turnovers and rapid divergence across lineages (Bachtrog et al., 2014; Kitano and Peichel, 2012). For aquaculture species such as snakeskin gourami, molecular investigations are essential to establish the underlying heterogametic system and to guide the design of reliable sex-control strategies.

Although extensive progress has been made in elucidating sex determination systems in diverse teleosts, the underlying mechanism in snakeskin gourami remains unresolved, highlighting the need for species-specific molecular investigations.

Table 2.2 Sex determination systems and identification approaches in teleosts.

No.	Sex Determination System	Identification Method	Species	Reference
1	XX/XY	FISH	Medaka (*Oryzias latipes*)	Matsuda et al. (2002)
2	ZZ/ZW	Linkage mapping	Tanganyika tilapia (*Oreochromis tanganicae*)	Cnaani and Kocher (2008)
3	$X_1X_1X_2X_2$ / X_1X_2Y	Cytogenetics; GWAS	Collichthys lucidus (*Collichthys lucidus*)	Cai et al. (2019)
4	ZZ/ZW	GWAS, SNP	Chilean Jack Mackerel (*Trachurus murphyi*)	Canales-Aguirre et al. (2025)
5	XX/XY	Genome Walking	Silver carp (*Hypophthalmichthys molitrix*)	Liu et al. (2018)
6	ZZ-ZW_1W_2	Molecular Cytogenetics	Lizardfish (*Synodontidae*)	Ueno et al. (2001)
7	$X_1X_1X_2X_2/X_1X_2Y$	Conventional Cytogenetics	Freshwater stingrays (*Potamotrygonidae*)	Cruz et al. (2011)
8	ZZ/ZW	GWAS	Determination of turbot (*Scophthalmus maximus*)	Martínez et al. (2021)
9	ZZ/ZW	Molecular marker	Clearhead icefish (*Protosalanx hyalocranius*)	Xing et al., (2021)
10	XX/XY_1Y_2	Conventional cytogenetics	Ancistrus sp.1 "Balbina"	De Oliveira et al. (2008)
11	$Z_1Z_1Z_2Z_2$ / $Z_1Z_2W_1W_2$	Conventional cytogenetics	Ancistrus sp.2 "Barcelos"	De Oliveira et al. 2008)
12	$X_1X_1X_2X_2$ / X_1X_2Y	Molecular cytogenetics (FISH)	Gasterosteus aculeatus (*Japanese Population*)	Kitano and Peichel (2012)
13	$X_1X_1X_2X_2/X_1Y_1X_2Y_2$	Conventional & Molecular Cytogenetics	Bunocephalus Coracoideus (*Aspredinidae*)	Ferreira et al. (2016)
14	ZZ/Z0	Molecular cytogenetics	Glass Knifefish (*Eigenmannia trilineata*)	Araya-Jaime et al. (2017)
15	XX/X0	Conventional Cytogenetics	Armored catfish (*Loricariidae: Hypostominae*)	Alves et al. (2006)

2.3 Molecular Markers for Sex Determination in Teleost Fishes

Molecular markers have fundamentally advanced the study of sex determination in fishes and provided practical tools for aquaculture. By enabling early and reliable identification of genetic sex, these markers facilitate broodstock management, allow the detection of sex-reversed individuals, and support the production of monosex populations without reliance on hormonal treatments (Mei

and Gui, 2015). Early studies primarily employed low-throughput molecular marker systems. For example, random amplified polymorphic DNA (RAPD), a PCR-based technique using arbitrary primers to amplify random genomic regions, was applied to identify sex-associated markers in common carp (*Cyprinus carpio*) (Chen et al., 2009). Amplified fragment length polymorphism (AFLP), which combines restriction enzyme digestion with selective PCR amplification to generate genome-wide polymorphic profiles, was successfully used in rainbow trout (*Oncorhynchus mykiss*) (Felip et al., 2005). In addition, simple sequence repeats (SSR), co-dominant markers based on short tandem DNA repeats with high polymorphism and reproducibility, have been applied for sex-linked marker identification in half-smooth tongue sole (*Cynoglossus semilaevis*) (Song et al., 2012). Although these approaches contributed to the initial discovery of sex-linked loci in several species, their broader application has been constrained by labor-intensive procedures, limited genomic coverage, and relatively low resolution.

The advent of next-generation sequencing (NGS) has revolutionized marker discovery, allowing genome-wide identification of SNPs and insertion/deletion polymorphisms (indels) (Mei and Gui, 2015; Yano et al., 2012). Reduced-representation sequencing methods such as RAD-seq and ddRAD have been particularly useful in non-model fishes, facilitating dense marker development at moderate cost (Andrews et al., 2016). Moreover, RNA sequencing (RNA-seq) has emerged as a complementary approach, especially in species with homomorphic sex chromosomes, by revealing sex-biased gene expression and coding SNPs that can serve as candidate sex-specific markers (Qian et al., 2014). Table 2.3 summarizes representative teleost species in which transcriptomic (RNA-seq) and/or genome-wide sequencing approaches have contributed to the discovery and validation of sex-specific DNA markers.

Transcriptome-based approaches have also been widely applied for SNP discovery and marker validation in reptiles and amphibians, demonstrating their effectiveness in identifying reliable genetic markers beyond teleost fishes. Although RNA-seq typically generates a large number of putative variants, subsequent filtering and validation can yield highly robust SNP markers suitable for genetic and breeding-related applications. Similar transcriptome-derived SNP strategies have been successfully implemented in livestock and plant species, further highlighting the broad applicability and reliability of this approach (Ma et al., 2016; Pierzchała et al., 2019).

Collectively, these findings indicate that transcriptome-based SNP discovery provides a powerful and versatile framework for genetic marker development, particularly in non-model species with poorly characterized genomes. Accordingly, SNP markers were selected in the present study as the primary strategy for developing reliable sex-specific DNA markers in snakeskin gourami.

In snakeskin gourami, transcriptomic analyses have revealed pronounced differences in gene expression between testes and ovaries, providing important insights into gonadal development and sexual differentiation (Boonanuntanasarn et al., 2020; Kabpha et al., 2023). Recent studies have further identified sex-biased gene expression patterns in both juvenile and adult gonads, highlighting candidate genes potentially involved in steroidogenesis, germ cell development, and gonadal differentiation pathways (Boonanuntanasarn et al., 2020; Jantawongsri et al., 2025). These findings suggest that transcriptomic resources offer a valuable foundation for exploring the genetic basis of sex differentiation in this species.

Importantly, SNPs located within sex-biased genes or in linkage disequilibrium with sex-determining regions represent promising molecular markers for genetic sex identification. However, transcriptome-based discovery alone is insufficient without systematic validation, as sex-biased expression may reflect developmental stage rather than underlying genetic sex. Therefore, distinguishing genetic sex–associated SNPs from stage-dependent expression patterns is essential for developing reliable and stable DNA markers applicable across developmental stages (Leet et al., 2013).

Despite increasing transcriptomic knowledge, integrated studies combining RNA-seq–based SNP discovery with marker validation in phenotypically confirmed individuals remain limited in *T. pectoralis*. A systematic approach involving SNP identification from gonadal transcriptomes, followed by validation using PCR-based genotyping in independent populations, is therefore required to establish robust sex-specific DNA markers and to clarify the genetic architecture underlying sex determination in this species (Zhao et al., 2019).

Table 2.3 Sex-specific molecular markers identified in teleost fishes using sequencing-based approaches.

No.	Species (Latin name)	Transcriptomic / Sequencing method	Type of molecular marker	Reference
1	Northern Snakeheads (*Channa argus*)	RNA-Seq + GWAS	Sex-specific SNP & InDel	Liu, H et al. (2024)
2	Hypophthalmichthys nobilis (*bighead carp*)	2b-RAD + genome-wide sequencing	Male-specific DNA marker	Liu, H et al. (2018)
3	silver carp (*Hypophthalmichthys molitrix*)	2b-RAD + genome-wide sequencing	Male-specific DNA marker	Liu, H et al. (2018)
4	Japanese meagre (*Argyrosomus japonicus*)	PacBio HiFi + Hi-C genome assembly + GWAS of sex	Sex-linked DNA markers	Liu, Y et al. (2024)
5	clearhead icefish (*Protosalanx hyalocranius*)	Whole-genome resequencing	Female-specific DNA marker (deletion)	Xing et al. (2021)
6	blunt snout bream (*Megalobrama amblycephala*)	WGS	Male-specific DNA marker	Fu et al. (2024)
7	channel catfish (*Ictalurus punctatus*)	Y-chromosome sequencing + comparative RNA-seq	Sex-specific isoform / DNA marker	Bao et al. (2019)
8	Crimson seabream (*Pargyrops edita*)	Transcriptomic (RNA-seq)	Simple sequence repeats (SSRs)	Shan et al. (2021)
9	European sea bass (*Dicentrarchus labrax*)	Small RNA-seq (miRNA sequencing)	sex-biased MiRNA markers	Van Gelderen et al. (2025)
10	darkbarbel catfish (*Pelteobagrus vachelli*)	GWAS and transcriptome	male sex-specific markers	Zhang et al. (2026)
11	Chinese tongue sole (*Cynoglossus semilaevis*)	Genome/Genotyping assay	Female-specific structural variation (Indel) & SNPs	Zhang et al. (2019)
12	yellow catfish (*Pelteobagrus fulvidraco*)	Transcriptomic (454 GS-FLX sequencing)	SNPs, SSRs, and INDELs from transcriptome	Chen et al., (2015)

2.4 Sex reversal and monosex breeding strategies in aquaculture

Sex reversal has long been applied in aquaculture as an effective strategy to generate monosex populations that maximize sex-specific growth advantages. Due to the pronounced sexual plasticity of teleost fish, sexual differentiation can be redirected during early developmental stages through endocrine manipulation, allowing to produce phenotypic males or females regardless of their genetic sex (Devlin and Nagahama, 2002). This biological flexibility provides the theoretical foundation for monosex breeding programs aimed at improving production efficiency, uniformity, and economic return.

At the physiological level, endocrine regulation plays a central role in directing gonadal differentiation and constitutes the mechanistic basis of artificial sex reversal.

Suppression of aromatase (cyp19a1a), the key enzyme responsible for estrogen biosynthesis, promotes testicular development, whereas elevated aromatase activity favors ovarian differentiation (Guiguen et al., 2010; Lambeth et al., 2013). Male-associated pathways involving anti-Müllerian hormone (Amh) and gonadotropins such as follicle-stimulating hormone (FSH) further modulate sex differentiation in a stage- and dose-dependent manner (Huang et al., 2019; Zhou et al., 2019). In addition, stress-related endocrine factors, including cortisol, may interact with these hormonal pathways and influence sexual outcomes under specific rearing conditions (Shen and Wang, 2014).

In practical aquaculture, sex reversal is commonly achieved through dietary or immersion treatments using steroid hormones, most notably 17β-estradiol (E2) for feminization and 17α-methyltestosterone (MT) for masculinization (Kabpha et al., 2023; Mu et al., 2024). Based on the mode of application, monosex production strategies are generally classified as direct or indirect. As illustrated in Figures 2.2 and 2.3, direct methods involve hormonal treatment of larvae during the labile period of gonadal differentiation, resulting in phenotypic males or females in the grow-out population. Indirect strategies, in contrast, rely on the generation of sex-reversed broodstock (neomales or neofemales), which are subsequently crossed with normal individuals to produce genetically uniform monosex offspring without hormonal exposure of the commercial generation.

Among these approaches, indirect monosex breeding has gained increasing attention because it mitigates concerns related to hormone residues, environmental contamination, and consumer acceptance. Figure 2.2 summarizes the pathways for monosex female production, in which androgen-induced neomales (phenotypic males with an XX genotype) are crossed with normal females to generate all-female progeny. Similar principles apply to all-male production systems using neofemales (Figure 2.3). These broodstock-based strategies enable sustainable monosex production while maintaining genetic stability across generations.

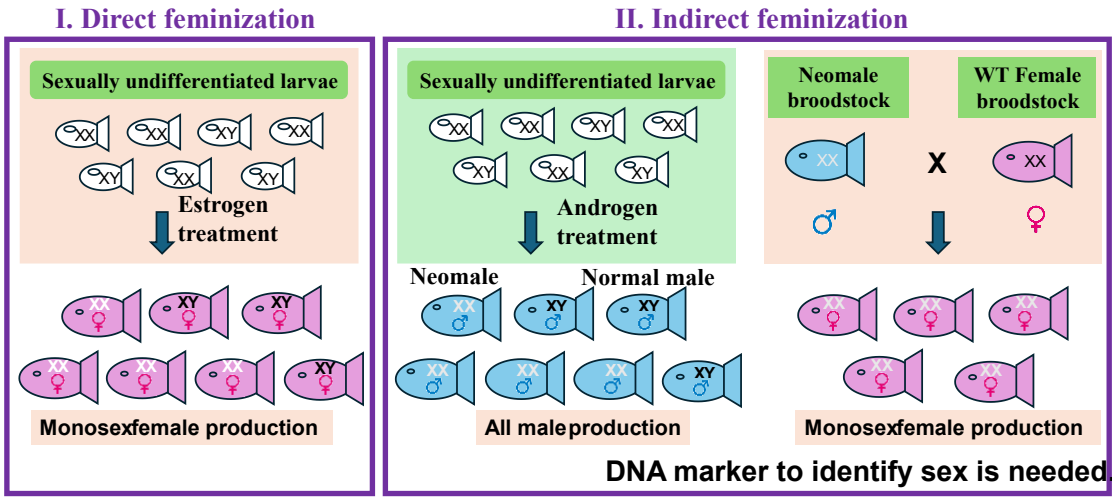

Figure 2.2 Schematic illustration of direct and indirect feminization strategies for monosex female production in fish.

The diagram illustrates two approaches for producing viable all-female populations. In direct feminization, sexually undifferentiated larvae are treated with estrogen, resulting in a phenotypically female population. In indirect feminization, androgen treatment during sexually undifferentiated larvae induces sex reversal, producing neomales that exhibit male phenotype but retain a female (XX) genotype. Neomale broodstock are crossed with wild-type females, leading to the production of monosex female offspring without hormonal treatment of the progeny. The figure highlights the use of early developmental stages and sex-reversed broodstock for female-biased aquaculture production.

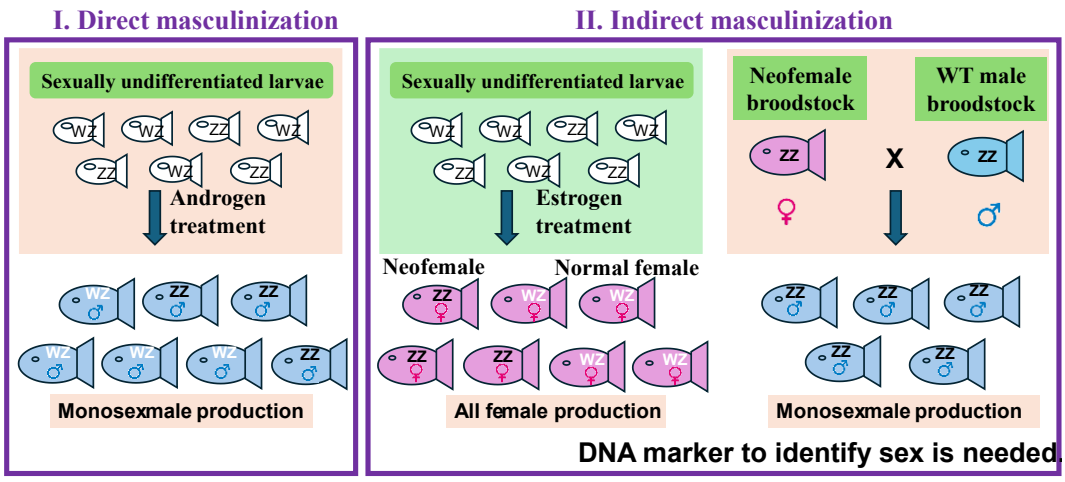

Figure 2.3 Schematic illustration of direct and indirect masculinization strategies for monosex male production in fish.

The figure shows the procedures used to obtain viable monosex populations through hormonal manipulation and broodstock-based approaches. In direct masculinization, sexually undifferentiated larvae are treated with androgen, resulting in phenotypic male populations. In indirect masculinization, estrogen treatment of the sexually undifferentiated larvae induces sex reversal, producing neofemales that exhibit a female phenotype but retain a male (ZZ) genotype. Neofemale broodstock are crossed with wild-type males, producing viable all-male offspring. The diagram highlights the use of early developmental stages and sex-reversed individuals for efficient monosex production in aquaculture.

The economic importance of monosex breeding is closely linked to sexual dimorphism in growth and performance traits. In many aquaculture species, including snakeskin gourami, half-smooth tongue sole (*Cynoglossus semilaevis*), largemouth bass (*Micropterus salmoides*), mandarin fish (*Siniperca chuatsi*), and sablefish (*Anoplopoma fimbria*), females generally exhibit faster growth and attain larger body size, making female-biased populations particularly advantageous (Bi et al., 2020; Kabpha et al., 2023; Luckenbach et al., 2017; Zhou and Chen, 2016). Conversely, male-biased growth has been reported in species such as northern snakehead (*Channa argus*), zig-zag eel (*Mastacembelus armatus*), and Nile tilapia (*Oreochromis* spp.), where all-male culture systems are preferred (Asad et al., 2024; Lu et al., 2024; Wang et al., 2019). Beyond

growth performance, sexual dimorphism may also affect ornamental traits, disease resistance, and stress tolerance, further shaping production strategies under intensive farming conditions (Eliason et al., 2020; Fjelldal et al., 2023; Morris et al., 2020).

Despite the proven effectiveness of sex reversal and monosex breeding, the long-term sustainability of these systems relies heavily on accurate identification of genetic sex and reliable selection of sex-reversed broodstock. Phenotypic observation alone is insufficient, particularly during early developmental stages or in species with homomorphic sex chromosomes. Consequently, the development of robust sex-specific DNA markers has become a critical component of modern monosex breeding programs. Such molecular tools enable precise genotypic sex identification, facilitate neomale or neofemale screening, and provide a foundation for hormone-free, marker-assisted breeding strategies, particularly in species such as snakeskin gourami, where female-biased growth offers clear production advantages.

2.5 References

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