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Research Article

Identification and Expression Analysis of Suppressors of Cytokine Signaling from Spotted Seabass (*Lateolabrax maculatus*)

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The suppressor of cytokine signaling (SOCS) plays a negative role in the cytokine signaling pathway, preventing excessive signaling from interfering with the metabolic homeostasis of the body. By regulating the Janus kinase-signal transducer and activator of transcription pathway through negative feedback, SOCS have a significant impact on the regulation of both innate and adaptive immunity against pathogens, thus playing a crucial role in the immune response, growth, and development of the body. In this study, the cDNA sequences of SOCS1, 2, 3a, 3b, 4, 5b, 6, 7, 8, 9, and CISH genes of spotted seabass (*Lateolabrax maculatus*), an important marine economic fish in China, were cloned using RT-PCR, nested PCR, and RACE techniques. Multiple sequence alignment showed that the SOCS family members shared highly conserved functional structural domains, including the SRC homology 2 domain (SH2 domain) and the SOCS-box domain. The phylogenetic analysis showed that SOCS1, 2, 3a, 3b, 8, and CISH belonged to the type II subfamily of SOCS genes, while SOCS4, 5b, 6, 7, and SOCS9 belonged to the type I subfamily. Furthermore, gene organization and syntenic analysis confirmed the phylogenetic analysis and protein annotation of the SOCS gene family in spotted seabass. Constitutive expression of spotted seabass SOCS genes was observed in various tissues of healthy fish, with varying expression levels. Following the lipopolysaccharide and *Edwardsiella tarda* challenge, the expression profiles of spotted seabass SOCS genes were differently regulated in the gill, head kidney, intestine, and spleen. These findings provide a basis for future research on the functional properties of SOCS genes in spotted seabass.

1. Introduction

Suppressors of cytokine signaling (SOCSs) are a class of intracellular proteins that are produced in response to cytokine action, which play an essential role in negative feedback regulation of cytokine-Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway, which prevent their overexpression [1]. The JAK-STAT signaling pathway mediates the intracellular signaling process of various cytokines and growth factors and activates the corresponding target genes, which are closely related to cell proliferation, differentiation, invasion, apoptosis, and immune regulation [2]. SOCS family proteins, acting as JAK-binding proteins or STAT inhibitors, which are regulated by different mechanisms, and there are four main mechanisms involved in the cytokine-JAK-STAT signaling pathway, including as follows: (a) blocking the binding of JAK to its substrate and inhibiting its activity; (b) inhibiting STAT phosphorylation; (c) binding to elongin BC and degrading the protein; (d) degrading JAK [2, 3]. Nowadays, there are eight SOCS proteins: SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6, SOCS7, and CISH have become the most significant cytokine receptor signaling inhibitors [4]. SOCS family members share a common structural feature consisting of a centrally located

SH2 domain and a conserved domain at the carboxyl terminus, known as the SOCS-box [5]. In addition, the N-terminal domain shows variability, and SOCS1 and SOCS3 have a specific small kinase inhibition region (KIR) within this domain [6, 7]. It is worth noting that SOCS has not only an inhibitory effect on signaling pathways but also an activating effect. In a recent study, it was found that redlip mullet socs5b could directly activate the PI3k/Akt pathway by itself, thus enhancing the proliferation and migration of cells [8].

In 1997, researchers identified eight members of the SOCS family, including SOCS1-7 and CISH, in mammals [9]. A decade later, Jin et al. [10] identified and characterized SOCS3b, SOCS5b, SOCS8, and SOCS9 in five model fish species, including Tetraodon, Zebrafish, Fugu, Stickleback, and Medaka, and systematically analyzed and identified them as new members of the SOCS family [11]. Phylogenetic analysis shows that the vertebrate SOCS gene family can be divided into two subfamilies: the type I subfamily consists of vertebrate SOCS4, SOCS5a, SOCS5b, SOCS6a, SOCS6b, SOCS7, and SOCS9. The type II subfamily is composed of CISH, SOCS1a, SOCS1b, SOCS2, SOCS3a, SOCS3b, and SOCS8 [12–14]. The way in which SOCS proteins are grouped together reflects their evolutionary history, with proteins that share similar structures and functions being clustered together [15].

To date, SOCS family members have been identified in a variety of fish species, in addition to the five model fish mentioned above, also including rainbow trout [16], channel catfish [17], tongue sole [18], nile tilapia [19], Japanese flounder [20], soiny mullet [5], yellow catfish [21], swamp eel [22], grass carp [14], and redlip mullet [8]. However, little is known about studies in Perciformes. This study will clone and identify the SOCS gene family and analyze their expression in eight tissues, i.e., brain, gill, head kidney, intestine, liver, muscle, skin, and spleen of spotted seabass (*Lateolabrax maculatus*). As the large-scale and intensive aquaculture for spotted seabass grows, pathogenic diseases have emerged as a crucial factor that limits its sustainable growth. Given the important role of SOCS family members in regulating immune signaling pathways, this study also aimed to observe their mRNA expression patterns at different time points following intraperitoneal injection of lipopolysaccharide (LPS) and *Edwardsiella tarda* stimulation in spotted seabass, hoping to further understand the functions of SOCS genes in spotted seabass and their evolutionary relationships.

2. Materials and Methods

2.1. Fish. All the spotted seabass used in this experiment were obtained from an aquaculture farm, Xiaoshan District, Hangzhou, Zhejiang Province, China. Before the experiment, a healthy spotted seabass with uninjured body surface, normal feeding, and active behavior, weighing 300 ± 50 g, was temporarily reared for one week in a barrel at a water temperature of 25°C, feeding once in the morning and evening. All the animal procedures were performed according to the instructions set by the Council of Animal Care of Shanghai Ocean University (SHOU-DW-2019-012).

2.2. Expression Analysis of Spotted Seabass SOCS after LPS and E. tarda Stimulation. The experimental fish were divided into three groups: The LPS experimental group (5 μg/g, 300 μL, intraperitoneal (ip) injection), *E. tarda* experimental group (1×10^4 CFU/mL, 300 μL, ip injection), and the control group (PBS, 300 μL, ip injection). Each group consisted of 40 fish, which were kept evenly and temporarily in four culture buckets. At 0, 6, 12, 24, and 48 hr after injection, four fish were randomly selected from each group for dissection. Four immune tissues (gill, head kidney, intestine, and spleen) were obtained from each fish. In addition, eight tissues (gill, head kidney, spleen, intestine, brain, skin, liver, and muscle) were collected from healthy fish. All the collected tissues were stored at −80°C.

2.3. Extraction of Total RNA, Preparation of cDNA, and qRT-PCR Template. Total RNA extraction was performed according to the instructions for the TRIzol method (Takara, Japan). The cDNA was synthesized using a SMART RACE cDNA Amplification Kit (Clontech, USA) for gene cloning. The Revert Aid™ First Strand cDNA Synthesis Kit (Fermentas, USA) was used to synthesize cDNA for quantitative real-time PCR (qPCR).

2.4. Design of Primers. The partial cDNA sequences of SOCS family genes were obtained by local Blast analysis using the BioEdit program through the genomic database (https://www.ncbi.nlm.nih.gov/genome/43909), and the sequences of closely related species were compared with those obtained by NCBI comparison, and DNAMAN software was used to design-specific primers in the relatively conserved regions. The fragments were compared with the sequences of closely related species obtained by NCBI and specific primers were designed in the relatively conserved region by DNAMAN software for cloning of partial cDNA fragments; the obtained fragments were sequenced, and RACE-specific primers were designed at both ends of the obtained target fragments by Primer 5.0 software. The primers used in this experiment were synthesized by Hangzhou Jinweizhi Biotechnology (Table S1).

2.5. Gene Cloning. The cDNA templates from eight tissues: gill, head kidney, spleen, intestine, brain, skin, liver, and muscle were mixed and diluted 20-fold for use as gene cloning templates. Spiking systems: Ex Taq enzymes (Takara, Japan) 12.5 μL; F primer 1 μL; R primer 1 μL; cDNA template 1 μL; ddH_2O up to 25 μL. Reaction procedures: 95°C 2 min; 98°C 10 s, 60°C 30 s, 72°C, 38 cycles; 72°C 7 min. Subsequently, the target bands were excised and recovered using OMEGA Bio's Gum Recovery Kit (OMEGA, USA). The recovered product was ligated with pMD-19T vector (Takara, Japan) overnight at 16°C. The ligation products were transformed into *Escherichia coli* DH5α competent cells. Single colonies were selected and sequenced by Shanghai Sangon Biotech Company.

2.6. Sequence Analysis. The spliced 5′-RACE, 3′-RACE, and cDNA fragments were assembled using DNAMAN software to obtain the full-length sequence of CISH, SOCS1, SOCS2, SOCS3a, SOCS3b, SOCS4, SOCS5b, SOCS6, SOCS7, SOCS8, and SOCS9. The open reading frames (ORFs) of the obtained sequences were predicted using the NCBI online website (https://www.ncbi.nlm.nih.gov/orffinder/). Homology matching

of the inferred amino acid sequences of the identified SOCS parent genes in the NCBI database was obtained using Protein BLAST at the NCBI online website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Protein molecular weights and isoelectric points of the corresponding amino acids were predicted using the Expasy online website (https://web.expasy.org/compute_pi/). The structural domains of the protein were determined using the SMART (http://smart.embl-heidelberg.de) online website. Exon–intron analysis of the gene was performed using the Ensembl online website (http://asia.ensembl.org/index.html). A neighbor-joining tree was constructed using the neighbor-joining method using MEGA X software, where the bootstraps value was set to 10,000. The upstream and downstream genes of the vertebrate SOCS gene are available on the Genomicus v108.01 online website (https://www.genomicus.bio.ens.psl.eu/genomicus-108.01/cgi-bin/search.pl).

2.7. qPCR Analysis. EF-1α was used as the internal reference gene. Quantitative real-time experiments were performed using the Light Cycle 480 system (Roche, Germany). The qRT-PCR reaction consisted of SYBR Green Master Mix (YEASEN) 5 μL, forward primer 0.2 μL, reverse primer 0.2 μL, and cDNA template 4.6 μL. The thermal profile for qRT-PCR was 95°C for 30 s, followed by 40 cycles of 95°C for 10 s, 60°C for 20 s and 72°C for 20 s.

2.8. Data Statistics. One-way analysis of variance (ANOVA) in IBM SPSS Statistics software was used for statistical ANOVA, with a difference level of "*" indicating a significant difference ($P<0.05$), and "**" indicating a highly significant difference ($P<0.01$). Histograms were plotted using GraphPad Prism 9.0 software, and data were expressed as mean ± SEM ($n=4$).

3. Results

3.1. Sequence Identification of the Spotted Seabass SOCS Family Genes. In this study, 11 genes of the SOCS family of spotted seabass, including CISH, SOCS1, SOCS2, SOCS3a, SOCS3b, SOCS4, SOCS5b, SOCS6, SOCS7, SOCS8, and SOCS9, were cloned using RT-PCR and RACE-PCR techniques. Sequence characteristics are shown in Table 1. Among them, the ORFs were 612, 744, 603, 618, 681, 1,200, 1,692, 1,611, 2,559, 645, and 1,644 bp, encoding amino acid sequence lengths of 203, 247, 200, 205, 226, 399, 563, 536, 852, 214, and 547 aa, respectively. The molecular weights (kDa) of the proteins predicted through the Expasy online website were 22.56, 27.37, 22.50, 23.24, 25.16, 45.40, 62.07, 59.85, 91.51, 24.05, and 61.22 kDa, respectively. The isoelectric points (*p*I) were 9.35, 9.16, 7.76, 8.95, 7.92, 9.06, 8.91, 6.09, 6.78, 8.30, and 6.77, respectively. The GenBank accession numbers on NCBI are nos. OQ540951, OQ540952, OQ540953, OQ540954, OQ540955, OQ540956, OQ540957, OQ540958, OQ540959, OQ540960, and OQ540961, respectively.

3.2. Phylogenetic Analysis, Sequence Similarity, and Amino Acid Characterization of the Spotted Seabass SOCS Family Genes. Amino acid structure mapping of spotted seabass SOCSs family members was performed using IBS Illustrator for Biological Sequence online mapping software (http://ibs.biocuckoo.org). It was observed that the spotted seabass SOCSs family members share two structural domains, the SH2 and SOCS-box domains, at the C-terminus. All type I members have longer amino acid sequences than type II members, with the variation concentrated in the N terminus. Using DNAman software, multiple sequence alignment was performed for type I and II subfamily members, revealing that the SH2 and SOCS-box domains of both types are conserved despite having differences in amino acid length (Figure 1).

To observe the evolutionary relationship of SOCS family genes in spotted seabass, a phylogenetic tree was constructed by the NJ-joining method with MEGA X software based on amino acid sequences SOCSs of spotted seabass and other representative vertebrates, including human (*Homo sapiens*), mouse (*Mus musculus*), zebrafish (*Danio rerio*), rainbow trout (*Oncorhynchus mykiss*), medaka (*Oryzias latipes*), tetraodon (*Tetraodon nigroviridis*), fugu (*Takifugu rubripes*), and stickleback (*Gasterosteus aculeatus*) (Figure 2). The results showed that the SOCS family genes formed two major branches, classified as type I and type II, according to Hong-Jian et al. [12] and Liongue et al. [13]. In the phylogenetic evolutionary tree, type I members, including SOCS4, SOCS5b, and SOCS9, cluster into one large branch, with SOCS5b and SOCS9 forming a smaller branch, while SOCS6 and SOCS7 cluster into a separate large branch. Type II members, SOCS1, SOCS3a, and SOCS3b, cluster into one large branch, with SOCS3a and SOCS3b forming a smaller branch, while CISH, SOCS2, and SOCS8 cluster into another large branch, with CISH and SOCS8 forming a smaller branch.

The SOCS amino acid sequences were further compared with those of representative vertebrates (Table 2). Results showed that all the SOCS genes, except for SOCS7, exhibited significant similarity with the SOCS genes of Stickleback, ranging from 76.31% to 93.84%). In the phylogenetic tree, the homology of SOCS1, SOCS2, SOCS3a, SOCS4, and SOCS8 with the spotted seabass SOCSs genes of the Stickleback ranged from 76% to 98%, indicating their high dependence on Stickleback. Meanwhile, SOCS6 showed significant similarity across species (ranging from 65.81% to 93.84%), suggesting that it has been conserved throughout evolution. In contrast, the remaining spotted seabass SOCSs genes exhibited lower similarity (ranging from 40.15% to 67.33%) with mammals (humans and mouse) but demonstrated higher similarity (ranging from 51.19% to 91.59%) with other fish species.

3.3. Gene Organization and Syntenic Analysis of Spotted Seabass SOCS Family Genes. The obtained SOCS gene sequences were put into through the perch genome data (https://www.ncbi.nlm.nih.gov/genome/43909) and analyzed using the BioEdit program to get the intron–exon information of the perch SOCS gene. The exon–intron structures of spotted seabass SOCS genes were compared with those of other species to assess their evolutionary conservation. Information on spotted seabass SOCSs was obtained from the spotted seabass transcriptome library and compared with information on this SOCSs gene for zebrafish, human, and mouse downloads. As shown in Figure 3, the results indicate that SOCS genes have a typical exon–intron structure

Table 1: The summary of spotted seabass SOCS family member features.

Sequence features	CISH	SOCS1	SOCS2	SOCS3a	SOCS3b	SOCS4	SOCS5b	SOCS6	SOSC7	SOCS8	SOCS9
GenBank accession numbers	OQ540951	OQ540952	OQ540953	OQ540954	OQ540955	OQ540956	OQ540957	OQ540958	OQ540959	OQ540960	OQ540961
5′UTR (bp)	244	278	256	193	11	—	162	124	72	—	258
3′UTR (bp)	468	447	1,375	1,153	242	1,697	183	—	—	706	253
ORF (bp)	612	744	603	618	681	1,200	1,692	1,611	2,559	645	1,644
Length of amino acids (aa)	203	247	200	205	226	399	563	536	852	214	547
Molecular weight (kDa)	22.56	27.37	22.50	23.24	25.16	45.40	62.07	59.85	91.51	24.05	61.22
Theoretical *p*I	9.35	9.16	7.76	8.95	7.92	9.06	8.91	6.09	6.78	8.30	6.77

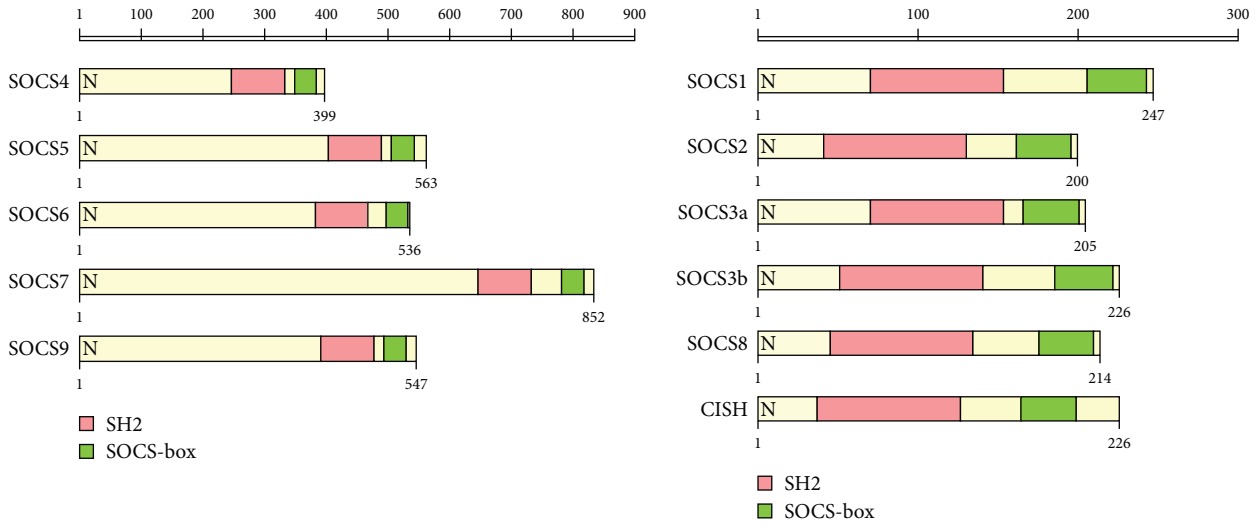


FIGURE 1: Functional domain of spotted seabass SOCS family proteins. SOCS4, 5b, 6, 7, and SOCS9 are type I subfamilies, and SOCS1, 2, 3a, 3b, 8, and CISH are type II subfamilies. Only the SH-2 and SOCS-box domains were predicted using SMART software, so the other domains are not labeled in the figure.

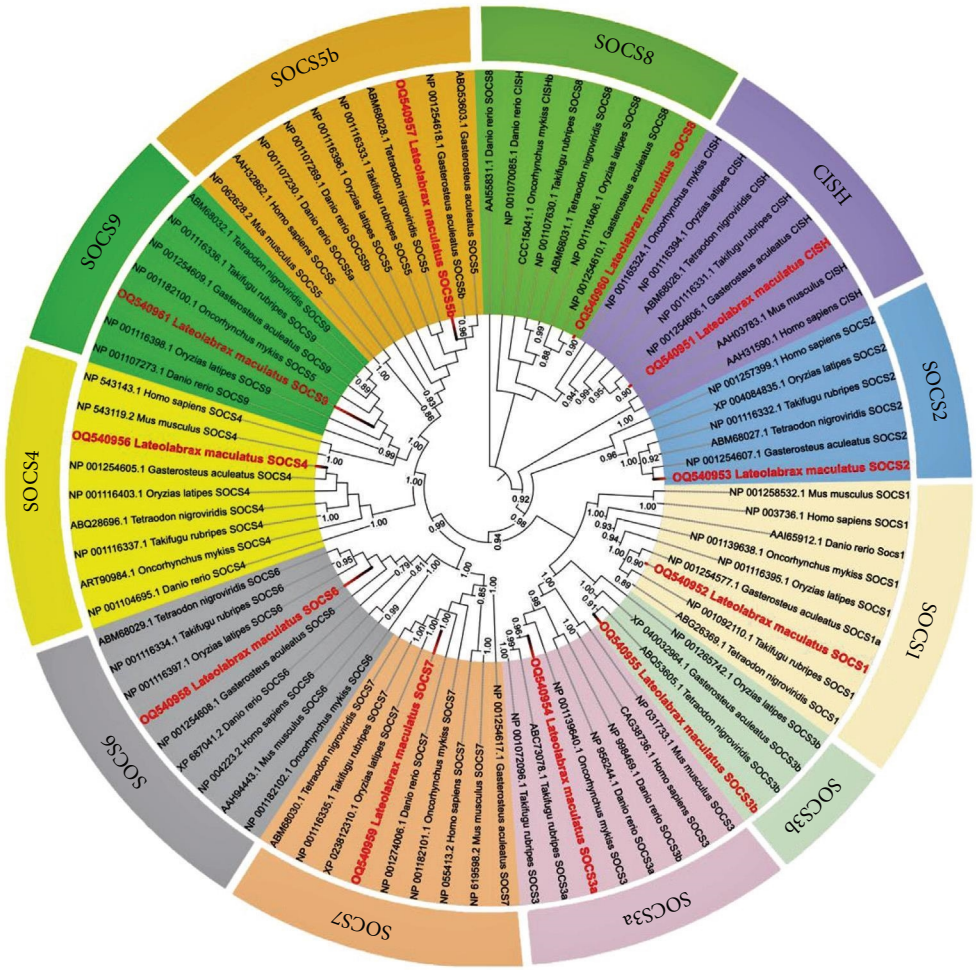


FIGURE 2: Phylogenetic tree of SOCS family genes from spotted seabass and other species. Deduced amino acid sequences of SOCS family members were aligned and the tree was constructed with the neighbor-joining method using the MEGA X software. The tree is bootstrapped 10,000 times. Accession numbers for sequences used are shown in Table S3.

Table 2: The sequence identity between seabass SOCS genes and those of other vertebrates.

L. maculatus	CISH (%)	SOCS1 (%)	SOCS2 (%)	SOCS3a (%)	SOCS3b (%)	SOCS4 (%)	SOCS5 (%)	SOCS6 (%)	SOCS7 (%)	SOCS8 (%)	SOCS9 (%)
H. sapiens	40.15	44.38	60.23	56.77	52.68*	67.92	65.60	71.22	67.33	47.32*	56.66*
M. musculus	44.45	44.38	59.54	56.77	52.66*	67.30	66.43	65.81	67.08	48.74*	56.66*
D. rerio	51.75	54.07	81.00	66.03	57.71	70.89	66.39	79.48	54.66	54.05	61.74
O. mykiss	69.47	53.15	80.60	77.36	54.50*	72.59	50.77	64.50	79.92	55.19*	77.30*
O. latipes	84.51	61.94	71.00	58.54	71.28	81.98	90.59	90.30	80.30	73.36	83.06
T. nigroviridis	81.07	75.54	81.09	83.90	81.11	78.48	90.16	90.49	83.53	66.36	79.42
T. rubripes	82.74	78.26	80.1	82.44	51.74*	85.82	87.81	90.86	85.21	64.49	76.29
G. aculeatus	91.59	76.31	80.50	83.41	81.19	83.08	91.31	93.84	51.19	78.50	52.33*

*Note: Since SOCS3b, SOCS8, or SOCS9 were not available in some species, other homologs were used instead, where SOCS3 was substituted for SOCS3b, CISH for SOCS8 and SOCS5 for SOCS9.

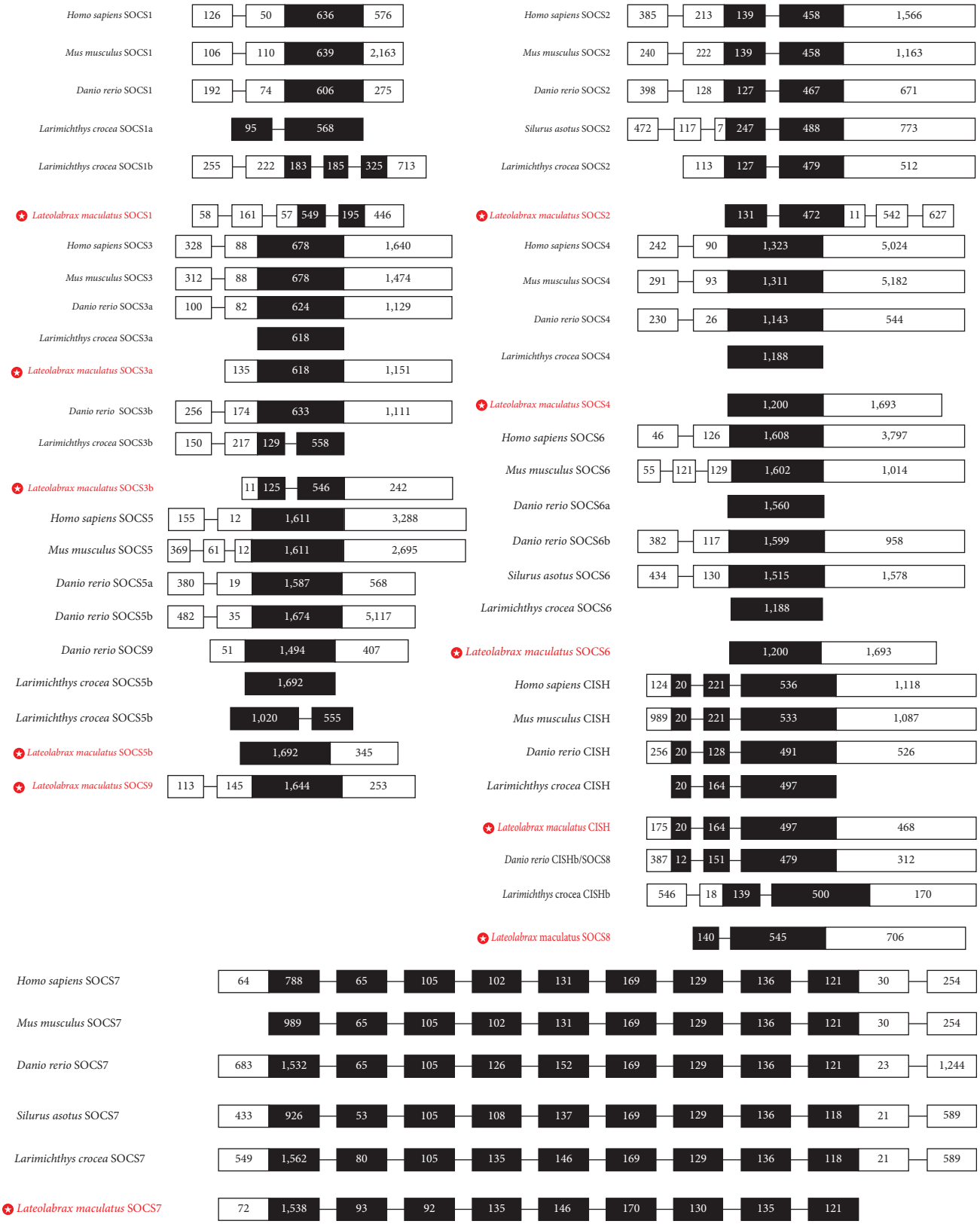


FIGURE 3: Genomic gene organization of spotted seabass SOCSs compared with other animals SOCSs. Spotted seabass SOCS3a and SOCS3b, SOCS5b, and SOCS9, and CISH and SOCS8 were analyzed together because of their structural specificity.

that is conserved across fish and mammal species. CISH shares a similar structure with zebrafish, human, and mouse suggesting that it likely performs a similar function in these species. The SOCS1 has a unique exon–intron structure compared to other species, indicating that it may have distinct functional characteristics. Furthermore, SOCS3a, SOCS4, SOCS5, SOCS6, and SOCS9 in all compared fish and mammals have a single exon, while all ORFs of SOCS7 have nine exons and eight introns, with each exon having a similar number of base pairs.

In order to gain a better understanding of how spotted seabass SOCS genes are related to those of other species, the genes were analyzed for linearity. As shown in Figures 4(a) and 4(b), spotted seabass SOCSs genes exhibit varying scaffold structures. The upstream genes of SOCS1 were relatively conserved in fish, with both the Cacng3b and Prkcbb genes being shared. Tnrc6a, a gene unique to Fugu and Stickleback, was not found in spotted seabass. The downstream genes in both mammals and fish, including the Clec16a gene and the Dexi gene, were relatively conserved. In contrast, the downstream motifs of SOCS7 were more similar only in fish, whereas the upstream motifs were more similar in both mammals and fish. Despite genetic differences between SOCS2, SOCS4, SOCS8, and CISH and human and mouse SOCS genes, their motifs are more similar in fish. Surprisingly, SOCS5 and SOCS9 were not identified in the spotted seabass transcriptome library, but SOCS5 was predicted to be located downstream of Rhoq on the seventh chromosome based on syntenic analysis of other species. No motif information was found upstream and downstream of spotted seabass SOCS9 in the library. SOCS6 is the most conserved of all the SOCS family genes in spotted seabass, being identical not only to other fish but also to human and mouse genes.

3.4. Expression Analysis of Spotted Seabass SOCS Genes in Healthy Fish. To investigate the tissue distribution of SOCS family genes in healthy spotted seabass, eight tissues were selected for QRT-PCR analysis. As shown in Figure 5, the mRNA expression levels of CISH, SOCS1, and SOCS3a showed the highest expression levels in the gill and relatively lower levels of expression in the brain, head kidney, intestine, and liver. SOCS2, SOCS3b, and SOCS8 also exhibited the highest expression in muscle and the lowest level in the head kidney. SOCS4 was predominantly expressed in the gill, intestine, muscle, skin, and spleen, with lower expression levels in the head kidney and liver tissues. SOCS5b, SOCS6, and SOCS7 exhibited a similar expression pattern with the highest expression levels in the brain, lower levels in the liver, and higher expression levels in other tissues. Conversely, spotted seabass SOCS9 had a different expression pattern from SOCS5b, with the highest expression observed in the skin and the lowest in the muscle.

3.5. Modulation of Spotted Seabass SOCS Genes in Response to E. tarda and LPS Injection for Gill, Head Kidney, Intestine, and Spleen. In order to study how spotted seabass SOCS family genes respond to the immune response, mRNA expression levels were examined in four immune tissues (gill, head kidney, intestine, and spleen) after injecting LPS and *E. tarda* at 0, 6, 12, 24, and 48 hr (Figures 6 and 7). The results showed that the mRNA expression patterns of various spotted seabass SOCS genes were different within the same immune tissue, and even the same SOCS gene displayed diverse expression patterns in different immune tissues.

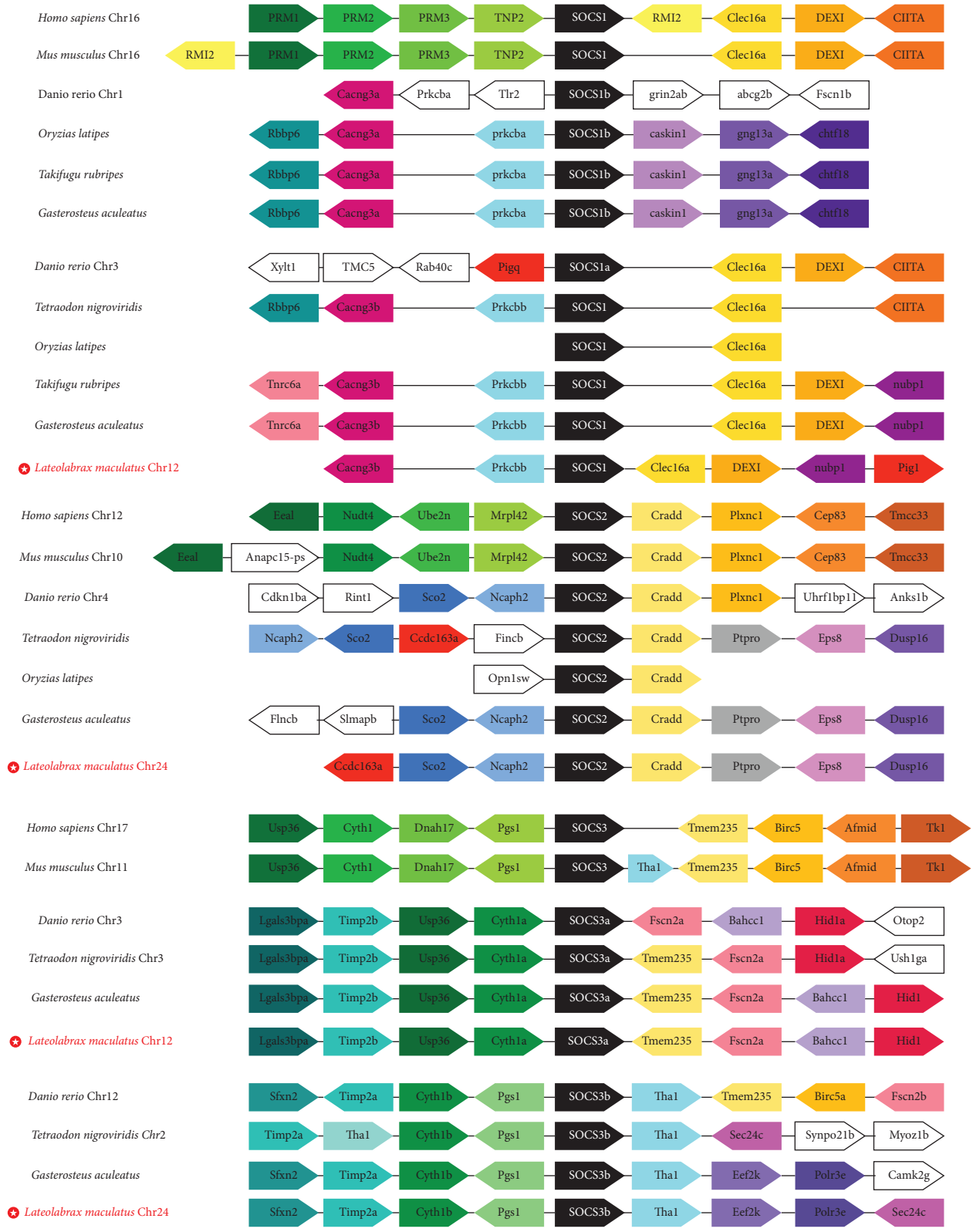
Surprisingly, the mRNA expression levels of the majority of spotted seabass SOCSs remained largely unchanged in the four tissues at any of the five time points sampled following the injection of *E. tarda*. SOCS3b, SOCS6, and SOCS8 in the spotted seabass demonstrated highly significant downregulation in individual immune tissues at the late injection stage ($P<0.01$) (Figures 6(6-d) and 7(3b-b, 3b-d, 8-c)). SOCS4 and SOCS5b showed significant downregulation, followed by significant upregulation adjustment (Figure 6(4-a, 4-b, 5-b)). SOCS5 was the only one that exhibited an extremely significant upregulation in gill and head kidney tissues at the late injection stage ($P<0.01$). Only SOCS9 showed highly significant downregulation in all immune tissues examined (Figure 7(9-a, 9-b, 9-c, 9-d)).

Following LPS injection, there was a significant variation in the mRNA expression levels of different the spotted seabass SOCSs genes at different time points, in contrast to *E. tarda* stimulation ($P<0.01$). In particular, most of spotted seabass SOCSs genes showed highly significant upregulation in the gill, head kidney, and spleen from the initial 6 hr of injection until 48 hr ($P<0.01$). However, in the head kidney and spleen of SOCS3, there was a highly significant upregulation in the preinfection period (6 hr), followed by a highly significant downregulation in the late infection period (24 hr) ($P<0.01$) (Figure 6(3b-b, 3b-d)).

4. Discussion

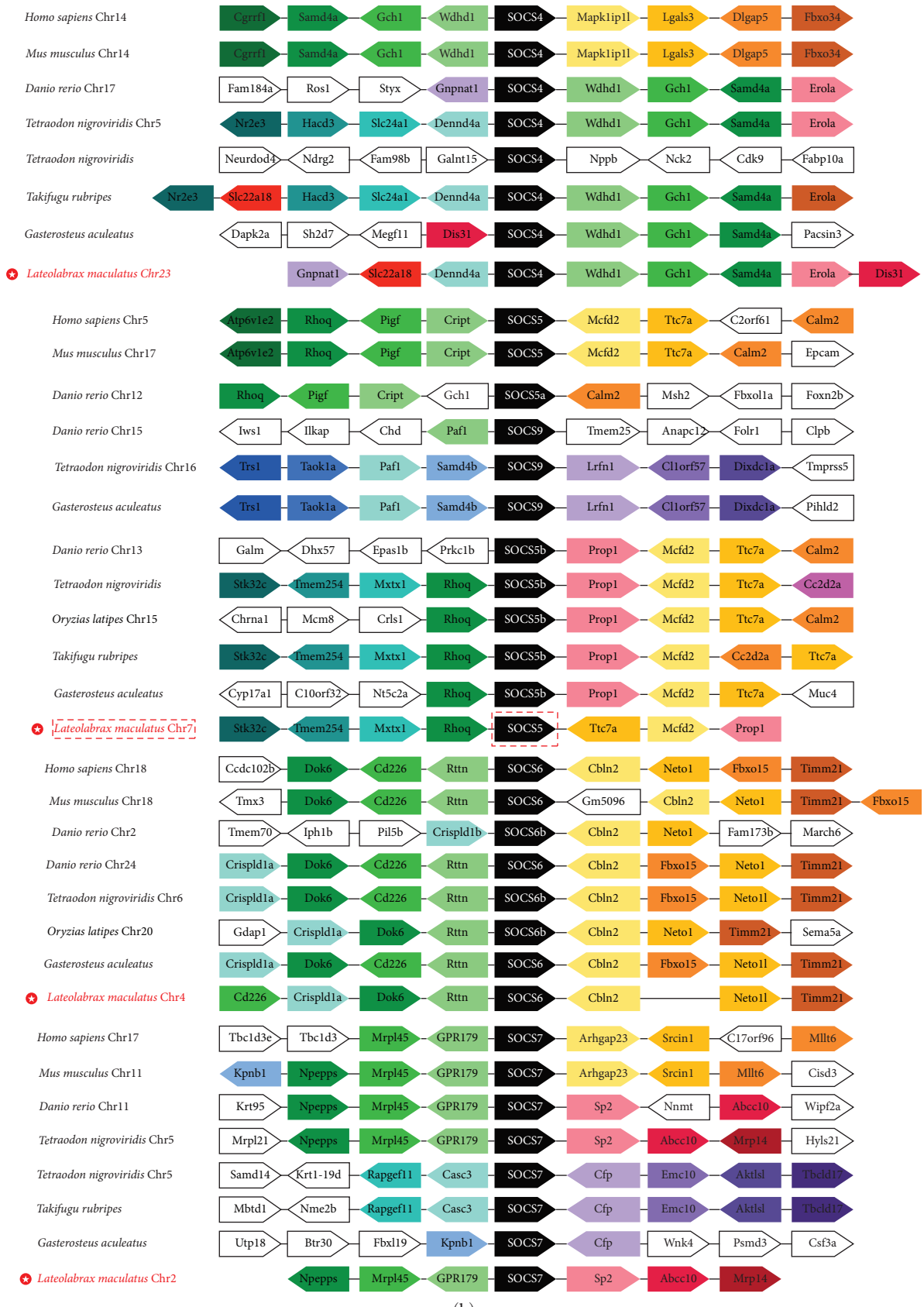
In the present study, 11 members of the spotted seabass SOCS family, including SOCS1-9, SOCS3b, and CISH, were cloned and identified from the spotted seabass. Notably, SOCS5b was also not found in the study of the swamp eel by Tian et al. [22]. Some studies suggest that SOCS9 and SOCS5 are produced by replication of the same gene, and therefore, SOCS9 is often referred to as SOCS5b [12]. For example, SOCS5b and SOCS9 are considered to be the same gene [5]. In the present study, we found evidence for the coexistence of SOCS5b and SOCS9 in spotted seabass, while further studies on SOCS5a in spotted seabass are needed.

SOCSs proteins with highly conserved motifs, such as the SH2 and SOCS-box structural domains, have been observed in various species [23]. The SH2 and SOCS-box domains, distinctive structural domains of SOCS proteins, are primarily responsible for inhibiting cytokine signals. The results of previous studies have shown that the SH2 domain and KIR binding to phosphopeptides and JAK2 domain, respectively, to achieve inhibition [24]. The SOCS-box has the ability to inhibit JAK kinase and identified a specific KIR domain near the N-terminal in SOCS1 and SOCS3 that allows them to maintain the ability to inhibit JAK kinase despite the absence of the SOCS-box domain [25]. In addition, the SH2 structural domain has the function of binding to the E2-E3 complex to ubiquitinate it [26]; the SOCS-box domain is involved in the formation of the E3 ligase complex, which promotes



(a)

Figure 4: Continued.



(b)

FIGURE 4: Syntenic analyses of spotted seabass SOCS family members compared with other animals SOCSs: (a) syntenic analyses of spotted seabass SOCSs type II subfamily members; (b) syntenic analyses of spotted seabass SOCSs type I subfamily members.

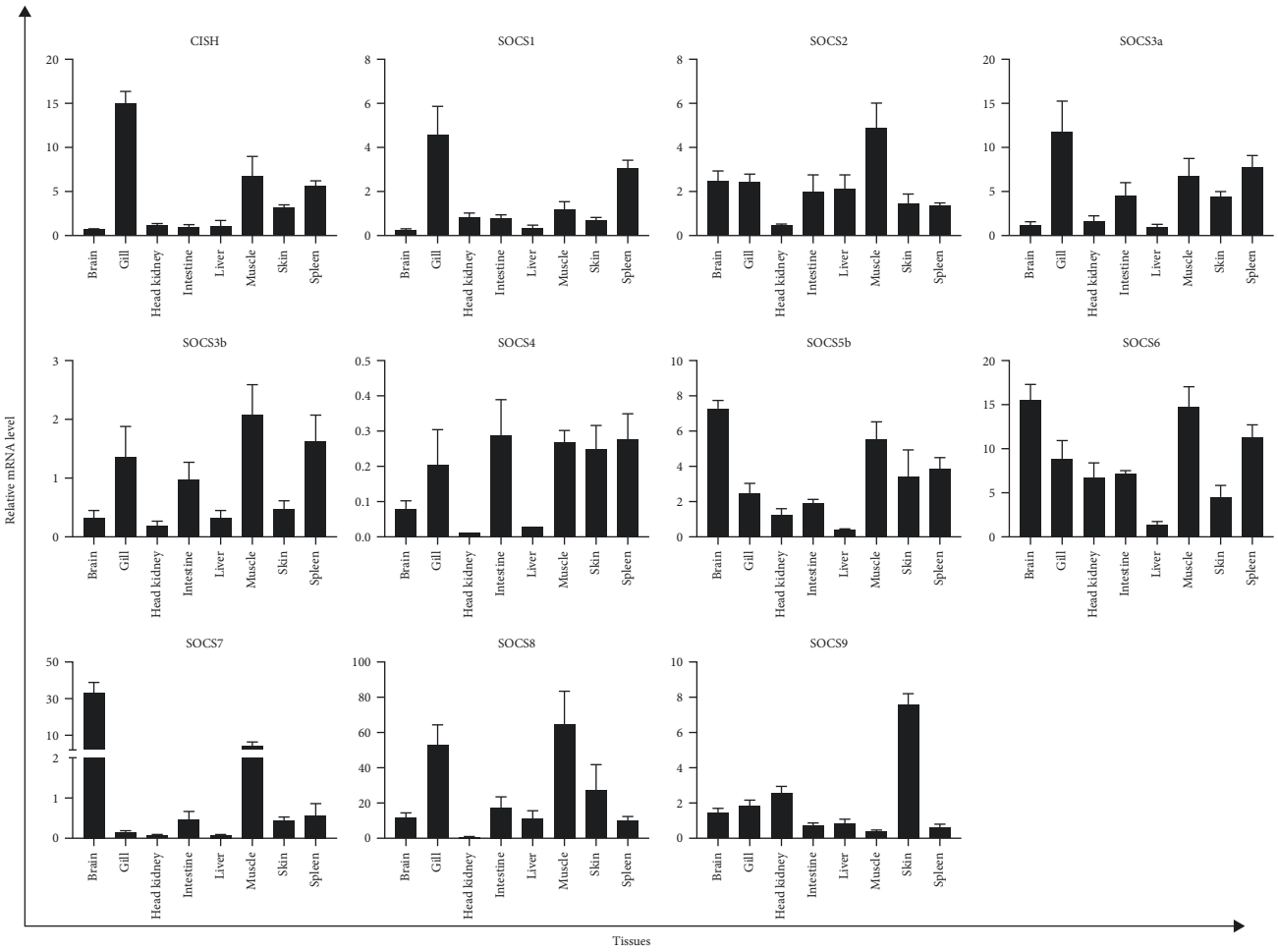


FIGURE 5: Expression pattern of spotted seabass SOCSs family genes in various tissues in healthy spotted seabass. Brain, gill, head kidney, intestine, liver, muscle, skin, and spleen were detected by qPCR. EF-1a was used as the reference gene, and the longitudinal column represented the mean ± SEM ($n = 4$).

degradation of the proteome by activating the cytokine receptor complex [27]. The functional, structural domains of spotted seabass SOCSs, including the SH2 and SOCS-box domains, were found to be highly conserved, similar to other fish studies. Moreover, researchers have identified a potentially conserved motif in the mouse SOCS gene family, which they named the PEST motif [28]. Subsequent research discovered PEST sequences in the SOCS genes of fish [21, 28]. However, the KIR and PEST motifs contained in spotted seabass SOCSs were not predicted using the SMART software; further studies are required to investigate the KIR and PEST motifs contained in spotted seabass SOCSs and their primary functions.

According to Van et al. [29], vertebrates underwent two rounds of whole-genome duplication (WGD) early in their evolution, resulting in the presence of 12 members of the SOCS gene family [30–32]. After the third WGD, the number of members increased to 15 members [16]. Wang et al. [33] suggested that these additional members may have arisen from species-specific gene duplication events. Currently, all SOCS genes after the third replication in rainbow trout are known to have undergone the fourth WGD, of which 26 SOCS genes were expressed [33] (Table S2). In recent research, it was observed that SOCS1 and SOCS3 of the hybrid yellow catfish "Huangyou-1" (♂*Pelteobagrus vachelli* × ♀*P. fulvidraco*) clustered together in a single branch [34], similar to the phenomenon observed in Soiny Mullet [5], and the high degree of homology between genes suggests the possibility of similar functions. The SOCS genes in fish are highly conserved with other higher vertebrates, particularly with SOCS5 and SOCS6, which show over 60% homology with other higher vertebrates [11]. The same situation was found in the spotted seabass. It is also worth mentioning that the spotted seabass SOCSs genes exhibit the highest overall homology with the SOCSs gene of the Stickleback based on the phylogenetic evolutionary numbers, indicating that the spotted seabass and Stickleback may have originated from the same ancestor.

SOCSs in spotted seabass have a typical exon–intron structure. The position of all spotted seabass SOCS genes on the locus, the order of upstream and downstream genes, and exon–intron structure similar to SOCSs in other species [35]. For example, spotted seabass SOCS1 and SOCS3a are found on the same chromosome (chromosome 12), and grass carp SOCS1 and SOCS3a were also on chromosome 2 [14], suggesting a close functional relationship between SOCS1 and

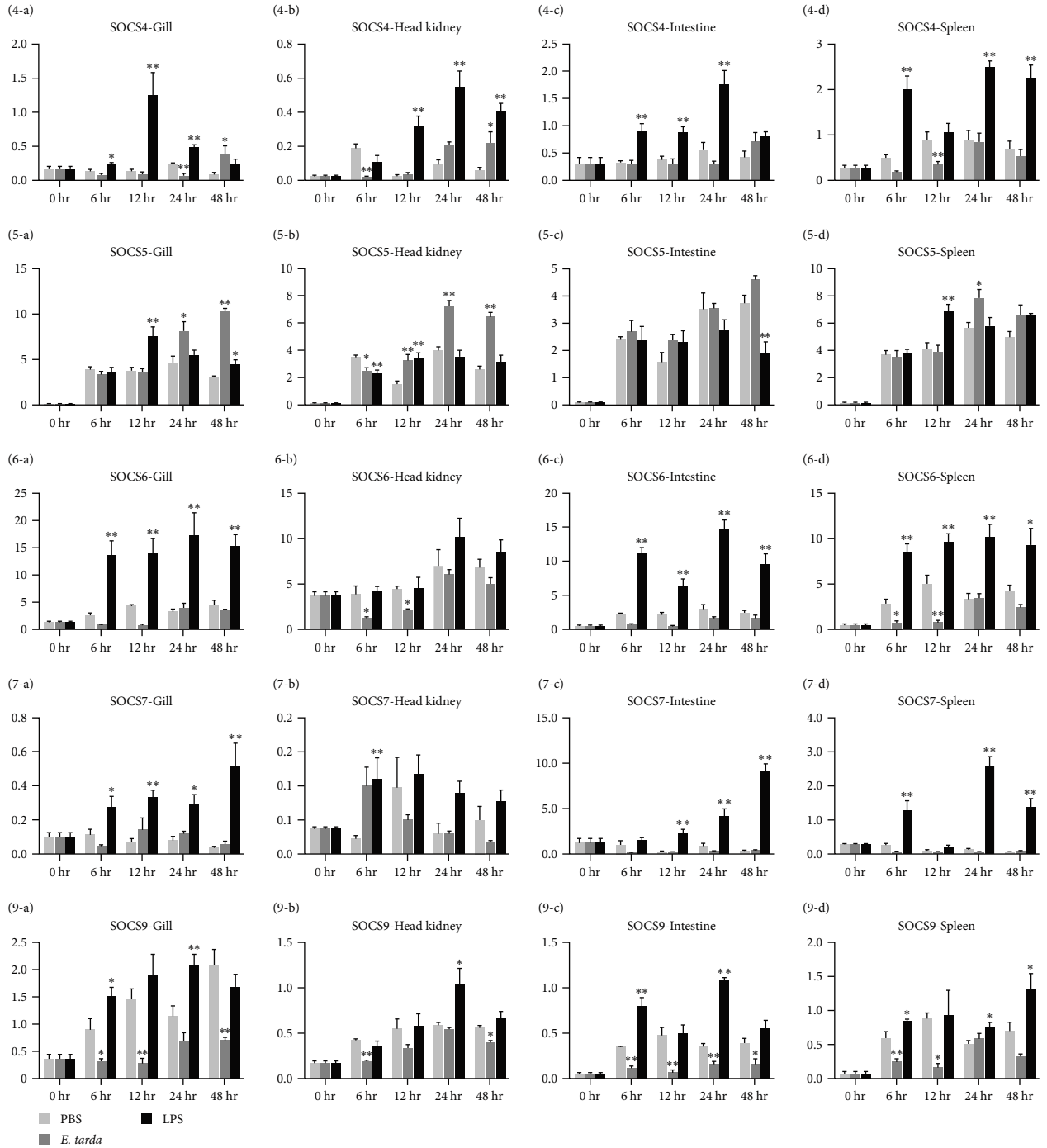


FIGURE 6: Expression of spotted seabass SOCS type II subfamily members following *E. tarda* and LPS infection. SOCS type II subfamily members' expression in the gill, head kidney, spleen, and intestine of spotted seabass was determined by qPCR at 0–48 hr after infection with *E. tarda* and LPS. At each time point, EF-1α was used as the reference gene. Value is expressed as the mean $\pm$ SEM ($n = 4$), $^{**}P < 0.01$, $^{*}P < 0.05$.

SOCS3a. However, further investigation is needed to determine whether the functions of other closely located genes, such as SOCS2 and SOCS3b (chromosome 24) and SOCS7 and CISH (chromosome 2), are similar. The majority of spotted seabass SOCSs genes have 1–3 exons, except for SOCS7, which is also found in all other fish [11, 14, 20, 36]. Therefore, it can be concluded that the gene structure of SOCSs in fish is highly conserved throughout the course of evolution. Meanwhile, this also suggests that the addition and deletion of exons in SOCS genes during genetic evolution may have contributed to their functional diversification. The gene structure of spotted seabass SOCSs is highly conserved in evolutionary terms, both compared to lower fish and higher vertebrates, indicating their biological importance.

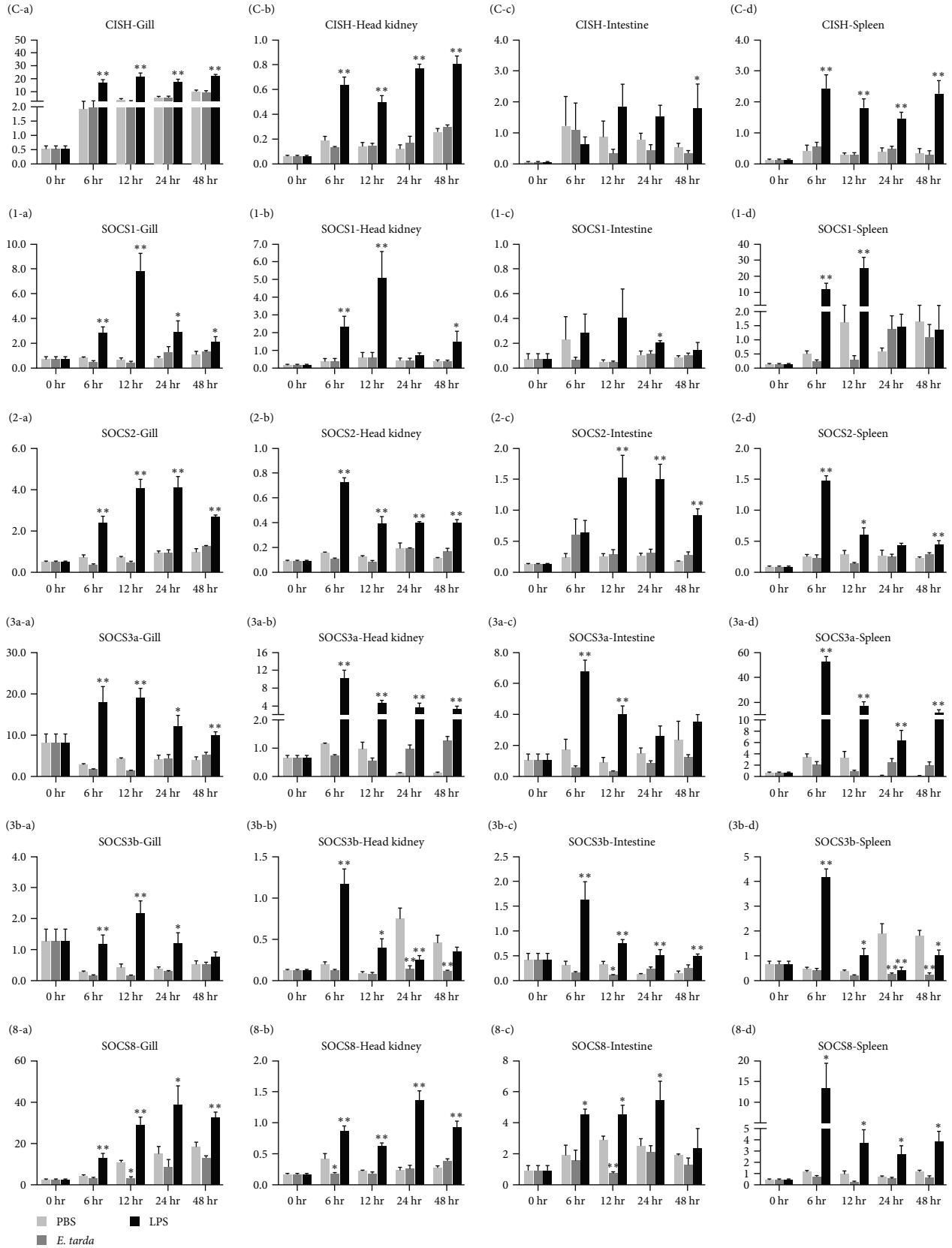


FIGURE 7: Expression of spotted seabass SOCS type I subfamily members following *E. tarda* and LPS infection. SOCS type I subfamily members' expression in the gill, head kidney, spleen, and intestine of spotted seabass was determined by qPCR at 0–48 hr after infection with *E. tarda* and LPS. At each time point, EF-1α was used as the reference gene. Value is expressed as the mean $\pm$ SEM ($n = 4$), $^{**}P < 0.01$, $^{*}P < 0.05$.

Gene linearity analysis of the neighboring genes of the spotted seabass SOCS genes with humans, mice, and five model fish species revealed that, though these genes differ greatly in humans and mice, the upstream and downstream genes of SOCSs in spotted seabass are highly similar to those of other fish species, suggesting their evolutional conservation in fish. There are two genes for SOCS3 and SOCS5 in fish, SOCS3a and SOCS3b, SOCS5a and SOCS5b, respectively. This implies that more replication events might have occurred during gene duplication in fish SOCS3 and SOCS5, leading to the production of multiple genes within a taxon [37]. In the present study, spotted seabass SOCS3 has also been verified to possess SOCS3a and SOCS3b, both SOCS3a and SOCS3b have different genes on the locus, but they are clearly related to human and mouse SOCS3, which share structural similarities with the SOCS3a and SOCS3b genes in zebrafish, confirming that SOCS3a and SOCS3b arose from the duplication of SOCS3 during the evolutionary process. The SOCS5b was identified in this study, showing a structural resemblance to SOCS5b in various fish species. This is direct evidence that we obtained the SOCS5b gene from the spotted seabass. However, there is no evidence to suggest that the SOCS9 obtained in this experiment can be used as a message for SOCS5a. Previous analyses have suggested that SOCS family members may have originated from a single ancestral gene through specific gene duplication, which is further supported by linearity analysis that clarifies homologous relationships and bridges gaps in phylogenetic evolutionary trees.

The various members of the SOCSs in spotted seabass exhibited diverse expression patterns across the eight selected tissues. CISH, SOCS1, and SOCS3a in spotted seabass displayed expression patterns similar to those of SOCS1 and SOSC3 in Nile tilapia [19] and SOCS1 in yellow catfish [34], with the highest expression levels observed in the gill. The gill are the primary sites of pathogen invasion in fish, and they contain IgT, which plays a crucial role in pathogen-specific immune responses following exposure [38]. This suggests that CISH, SOCS1, and SOCS3a likely play crucial roles in the innate and molecular immunity of spotted seabass. In contrast, SOCS2, SOCS3b, and SOCS8 had the highest expression levels in the muscle. It has been demonstrated that SOCS2 has a dual regulatory effect on the growth hormone (GH) signaling pathway, promoting the pathway at high levels of expression and inhibiting it at low levels [39, 40]. Experimental results in other fish have shown different expression patterns for the same genes. For example, the swamp eel has higher expression levels of SOCS2 in the head kidney and brain, but lower expression in muscle, while both fish species have similar expression patterns for SOCS3b [22]. This indicates that even for the same gene, expression patterns can vary between different species. Furthermore, studies have found that the expression level of SOCS7 affects GH signaling, which may explain its high expression in muscle tissue [41]. SOCS5b, SOCS6, and SOCS7 in spotted seabass showed the highest expression levels in the brain, indicating their potential role in regulating the central nervous system. SOCS4 in spotted seabass exhibited high expression levels in the skin and muscle, indicating their involvement in negative feedback regulation of epidermal growth factor receptor (EGFR) signaling [42]. SOCS8 is often referred to as CISHb and SOCS9 as SOCS5b in teleost fishes [5, 30]. However, the functions of these two genes require further investigation to better understand their biological significance.

After *E. tarda* stimulation, spotted seabass SOCS4 demonstrated a similar expression pattern in the gill, head kidney, and intestine, and all three tissues showed upregulation at 48 hr. In contrast, the spleen showed a downregulation after injection. This is unlike SOC4 expression in the Tongue sole, where the spleen showed upregulation at 48 hr but downregulation in other tissues [18]. SOCS7 in Tongue sole was also upregulated by *E. tarda*, but it showed a decrease at 12 hr following *V. harveyi* infection [18], suggests that different stimuli may have opposite results for the same gene in the same species. Of all the 12 SOCS genes in channel catfish, only SOCS1a, SOCS3a, and CISH were observed to be upregulated during the early phase after *E. ictaluri* infection [17], whereas SOCS6 and SOCS9 were upregulated in the spleen 12 hr after *E. tarda* injection, with SOCS5 and SOCS6 being downregulated after 3 hr in Japanese flounder [20]. In our study, we observed a significant or considerable downregulation of SOCS6 and SOCS9 mRNA expression in the spleen at 12 hr postinjection. These findings indicate that the expression of SOCSs in teleost fish may be species-specific and time-dependent. Notably, SOCS5b and SOCS9, being homologous genes, showed different expression patterns. This suggests that the occurrence of gene duplication events during evolution has led not only to structural changes in genes but also to functional differences.

Following LPS stimulation, most of the spotted seabass SOCS family members were observed to be upregulated in the four immune tissues at all time points from 6 hr postinjection compared to the control group injected with PBS. Similarly, in other studies, all members of the pike SOCS family were highly expressed after stimulation by *S. dysgalactiae* [5]. Additionally, Japanese flounder exhibited significant mRNA elevations for SOCS1, SOCS3, SOCS5, SOCS6, and SOCS5b during the early stages of bacterial infection [20]. Our results suggest that SOCS proteins have a dual function in bacterial-induced inflammatory response, exhibiting a prolonged and early response. Furthermore, these results imply that spotted seabass SOCS genes have distinct roles in regulating immune responses during bacterial infections with varying mechanisms. The substantial reduction in SOCS5b expression after stimulation was attributed to its elevated expression in the brain tissue, indicating its possible involvement in regulating neural centers and inhibition of diverse neural activities in response to infection. However, the function and mechanism of SOCS5b are still not well understood. Recent studies have shown that SOCS5b in redlip mullet (*Planiliza haematocheilus*) can inhibit viral hemorrhagic septicemia virus infection and EGFR expression but increase the expression of proinflammatory cytokines (IL-1β and IL-8) and antiviral genes (ISG-15 and IFN) when overexpressed [8]. Since most of spotted seabass SOCSs genes show an overall upregulation after LPS stimulation, suggesting that spotted seabass SOCSs are important regulators of immunity in spotted seabass.

Data Availability

Data for this research article are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Chuanguo Cai has done the methodology, writing—original draft preparation, and software. Jiasong Xie, Jiaqi Gao, Zhitao Qi, Ke Fan, Zhaosheng Sun, and Lina Lei have done the investigation, visualization, data curation, software, and validation. Qian Gao has done the writing—reviewing and editing, supervision, visualization, data curation, conceptualization, and methodology. All authors have read and agreed to the published version of the manuscript.

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Supplementary Materials

Table S1: primer used in the present study. Table S2: genes produced by rainbow trout at the time of each replication event. Table S3: NCBI accession numbers of the sequences used to construct the phylogenetic. (*Supplementary Materials*)

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