

Research Article

Identification and Expression Analysis of IL-10 Family in Spotted Sea Bass (*Lateolabrax maculatus*)

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Interleukin (IL)-10 family members play important roles in regulating the immune responses during host defense. In the present study, four IL-10 family members (IL-10, IL-20L, IL-22, and IL-26) were identified from spotted sea bass (*Lateolabrax maculatus*) and their expression patterns were investigated following *Edwardsiella tarda* and lipopolysaccharide (LPS) challenge. The four ILs of spotted sea bass shared conserved features of IL-10 family and were well clustered with the IL-10 family of fish, respectively. The expressions of these four ILs in normal tissues were different, but all were highly expressed in gills, indicating their roles in mucosal immunity. After *E. tarda* and LPS challenge, the four ILs were upregulated in several immune-related tissues (gills, head kidney, intestine, and spleen). These results indicated that these four ILs involved in the antibacterial immune responses of spotted sea bass, providing basis for understanding the function and networks of IL-10 family members in fish.

1. Introduction

Cytokines are small and widespread peptides that play important roles in the cell signaling [1]. However, cytokines cannot directly cross the lipid bilayer into the cytoplasm and they must rely on binding with their receptors on the membrane to transmit signals from the cell membrane to the nucleus [2]. Cytokines can be secreted by immune or nonimmune cells upon stimulation and involve in life processes, such as immune response, inflammatory reaction, cell proliferation, and growth [3, 4]. Interleukins (ILs) are leukocytes-produced cytokines and affect a variety of biological processes in an autocrine or paracrine manner, including tissue growth and repair, the dynamic balance of hematopoiesis, and the multilayered defense of the host against pathogens [5]. Among these cytokines, IL-10 is an immunomodulatory factor with multiple biological functions. A number of IL-10 family cytokines have been gradually identified that share

similar genetic structure, similar primary and secondary protein structures, and share similar receptor complexes, with IL-10 [6]. Accordingly, the members of the IL-10 family include IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26 [7, 8].

Until now, the IL-10 family has been identified in some fish species, such as IL-10 of Nile tilapia (*Oreochromis niloticus*), spotted knifejaw (*Oplegnathus punctatus*) and puffer fish (*Fugu rubripes*) [9–11], IL-20 like (IL-20L) of snakehead (*Channa argus*) and grass carp (*Ctenopharyngodon idella*) [12, 13], IL-22 of rainbow trout (*Oncorhynchus mykiss*) [14], and IL-26 of grass carp [15]. Overall, current studies mainly focus on the fish IL-10, and little on other members in the IL-10 family [9, 10, 12, 15, 16]. Fish seems to lack IL-19. Among these reported IL-10 family cytokines, the naming of IL-20L seems to be controversial, as IL-20L was originally named as IL-34 in zebrafish [17], and was referred to IL-20L in the latter studies [12, 13, 18, 19]. Also, in some gene databases, IL-20L was named as IL-19L.

Spotted sea bass (*Lateolabrax maculatus*), as an economic fish with the characteristics of eurythermic and euryhalic, has become one of the most common culture fishes in the north and south coastal areas of China [20]. With the expansion of the cultivation scale of this species, the economic losses that caused by some bacterial diseases are becoming more and more serious, which hinder the development of the culture industry of spotted sea bass [21–23]. *Edwardsiella tarda*, a common pathogen that can cause edwardsiellosis, is a Gram-negative bacterium that can affect a wide range of hosts, including aquatic animals, amphibians, reptiles, and mammals [24–26]. The IL-10 family members are essential for maintaining creaturely homeostasis and provide effective protection against microbial infection [27–30]. However, studies on these immune-related genes of spotted sea bass are not enough. Exploring the IL-10 family members of spotted sea bass can not only enrich the data on the immune mechanism of fish, but also provide the basis for developing the strategies for disease control in fish.

2. Materials and Methods

2.1. Experimental Animals and Sample Collection. Healthy spotted sea bass (300 ± 50 g) were purchased from a fish farm in Hangzhou city, Zhejiang Province, China, and kept under laboratory conditions at $26 \pm 2^\circ\text{C}$ for at least 7 days before the start of the experiment. To investigate the expression of IL-10 family in healthy spotted sea bass, eight tissues representative in the pathophysiology of vertebrates, including head kidney (HK), spleen, skin, gills, intestine, brain, liver, and muscle, were collected, quickly placed in dry ice, and then stored in -80°C refrigerator until use. All experiments were conducted in accordance with the Regulation for the Management of Laboratory Animals and the Guidelines for the Use of Research Animals of Shanghai Ocean University.

2.2. RNA Extraction and cDNA Acquisition. Total RNA was extracted from each tissue of spotted sea bass by using Trizol reagent (Invitrogen, USA). Then, the cDNA template was synthesized by reverse transcription kit (Takara, China) followed by the protocol as described previously [31, 32].

2.3. Cloning of IL-10, IL-20L, IL-22, and IL-26 from Spotted Sea Bass. Based on the homologous genes of IL-10 and IL-20L of *Perciformes*, gene-specific primer (GSP) of the IL-10 and IL-20L for spotted sea bass was designed by using the Primer 5.0 program, and the full length of the genes was subsequently obtained using the previous method [31]. Partial gene sequences of IL-22 and IL-26 of spotted sea bass were obtained from the transcriptomic data of spotted sea bass (unpublished). The cloning method is consistent with the other two genes. All primers used in gene cloning have been categorized in Table 1.

2.4. Sequence Analysis. Nucleotide and protein sequence analysis of the IL-10 family of spotted sea bass was performed through the NCBI website (<https://www.ncbi.nlm.nih.gov/>).

The phylogenetic tree and multiple sequence alignment of the IL-10 family genes of spotted sea bass were analyzed using the Clustal W, GeneDoc, and MEGA 5.1 programs, following the methods as described previously by Li et al. [31]. Signal peptides of protein sequence were predicted by the SignalP 5.0 (<https://services.healthtech.dtu.dk/services/SignalP-5.0/>). Gene syntenic relationships were analyzed using the Ensembl website (<https://asia.ensembl.org/index.html>) as previous studies on the IL-10 family [15, 33–35]. The NCBI genome databases, BioEdit and UltraEdit-32 programs, were used to analyze IL-10 family gene organizations in spotted sea bass.

2.5. Collection of Tissue Samples from Healthy Spotted Sea Bass. Total RNA from eight tissues was extracted and it was reversed into cDNA for quantitative real-time PCR (qPCR) by using Hifair™ II First Strand cDNA Synthesis SuperMix for qPCR (Yeasen, China). GSP was designed based on the highly conserved region of the IL-10 family nucleobase sequence, with amplification products ranging from 100 to 300 bp. A pair of primers for the housekeeping gene (elongation factor-1 α , *Ef1 α*) was designed to serve as a control for cDNA quantity and quality. The qPCR was performed using Hieff UNICON® q-PCR SYBR Green Master Mix (Yeasen, China) according to the product instructions. All primers are summarized in Table 1.

2.6. Challenge with Stimulation In Vivo. For the challenge experiment, 105 healthy spotted sea bass were equally divided into three groups. *E. tarda* (1×10^4 colony-forming units (CFU)/mL), lipopolysaccharide (LPS, 1 mg/mL), or phosphate-buffered saline (PBS) as control was injected with 300 μL into the intraperitoneal of the spotted sea bass, and challenge tests were performed as previously mentioned by Li et al. [31] and Sun et al. [36]. Then, tissues were taken at 6, 12, 24, and 48 hr after injection. At each time point, five test fishes were taken from each group to collect tissue samples. Total RNA was reverse-transcribed into cDNA for qPCR analysis. LPS was purchased from Sigma-Aldrich (USA), and *E. tarda* was prepared according to the previous study [31].

2.7. Statistics Analysis. Data from this study were analyzed using the independent samples *t*-test in the SPSS package 20.0 (SPSS Inc., Chicago, IL, USA). * $P < 0.05$ and ** $P < 0.01$ were considered statistically significant. The figures in this study were made by GraphPad Prism 5 software.

3. Results

3.1. Sequence Analysis of IL-10 Family Members of Spotted Sea Bass. The four sequences of IL-10 family in spotted sea bass were submitted to the GenBank database: OR051039 (IL-10), OR051040 (IL-20L), OR051041 (IL-22), and OR051042 (IL-26).

The full cDNA sequence of IL-10 of spotted sea bass was 1,126 base pair (bp), containing 162 of 5'-untranslated region (UTR), 400 bp of 3'-UTR, and 564 bp of open reading frame

TABLE 1: Primers used in this study.

Primer name	Sequence (5'-3')	Purpose
<i>Lm</i> IL-10-F	ATGACTCCTCGGTCTCTCCTCC	Partial cloning
<i>Lm</i> IL-10-R	GAAGCCAGATATGTCTCAATGTAGT	Partial cloning
<i>Lm</i> IL-20L-F	GCTGCTTGGCTGCTCTCTCTG	Partial cloning
<i>Lm</i> IL-20L-R	TGATAAACTCAGCATGCAGGGAG	Partial cloning
<i>Lm</i> IL-22-F	ACTCCTGCCGCTGCTTCTGA	Partial cloning
<i>Lm</i> IL-22-R	TGCAGGTACGTGAACAGGATGTC	Partial cloning
<i>Lm</i> IL-26-F	CGAGCTGATCCGAGACCTGTG	Partial cloning
<i>Lm</i> IL-26-R	GCTGTGCCAGTTCATCAATCCA	Partial cloning
<i>Lm</i> IL-10-5R1	AATGCTGTTTCATGGCGTGGC	5'-RACE
<i>Lm</i> IL-10-5R2	GGTTGTCGTTTGCCTCGTAGA	5'-RACE
<i>Lm</i> IL-10-5R3	CCAGGACGGACAGGAGGAGA	5'-RACE
<i>Lm</i> IL-10-3F1	CTCCTTCAAACTCCGTTCCG	3'-RACE
<i>Lm</i> IL-10-3F2	CTCAAGAGTGATGTCACCGACTGT	3'-RACE
<i>Lm</i> IL-10-3F3	ATGGGCGAACTGGATCTGCT	3'-RACE
<i>Lm</i> IL-20L-5R1	CATAGAAACGCAGCACAAAGACG	5'-RACE
<i>Lm</i> IL-20L-5R2	CGTATGGCGGAGTAATGTTTGC	5'-RACE
<i>Lm</i> IL-20L-5R3	CATTTGATCGTATGGCGGAGTA	5'-RACE
<i>Lm</i> IL-20L-3F1	CTCCGCCATACGATCAAATGC	3'-RACE
<i>Lm</i> IL-20L-3F2	GCGTCTTGCTGCTGCGTTTCT	3'-RACE
<i>Lm</i> IL-20L-3F3	TCTCAGCCAGAGCAGCAACG	3'-RACE
<i>Lm</i> IL-22-5R1	CGTGGTGATGATCGTGGTAGTG	5'-RACE
<i>Lm</i> IL-22-5R2	TCAGGTAGTAGTCGAGGATGTTGG	5'-RACE
<i>Lm</i> IL-22-5R3	GGCATCAGTCTGGTGCTGGA	5'-RACE
<i>Lm</i> IL-22-3F1	GAGGACGACTCCAGCACCAGAC	3'-RACE
<i>Lm</i> IL-22-3F2	CACGCCAACATCCTCGACTAC	3'-RACE
<i>Lm</i> IL-22-3F3	TGACAACACCCATCCCAGCAT	3'-RACE
<i>Lm</i> IL-26-5R1	GCTGTGCCAGTTCATCAATCCA	5'-RACE
<i>Lm</i> IL-26-5R2	AGCAGGTCATTGTAGTGAGGGG	5'-RACE
<i>Lm</i> IL-26-5R3	GCATCTCCAGCCAGCCAATC	5'-RACE
<i>Lm</i> IL-26-3F1	GATTGGCTGGCTGGAGATGC	3'-RACE
<i>Lm</i> IL-26-3F2	CAATGACCTGCTGTACCGACTG	3'-RACE
<i>Lm</i> IL-26-3F3	AGGTGGATTGATGAACTGGCACA	3'-RACE
UPM-long	CTAATACGACTCACTATAGGGCAAG CAGTGGTATCAACGCAGAGT	3'-RACE
UPM-short	CTAATACGACTCACTATAGGGC	3'-RACE
NUP	AAGCAGTGGTATCAACGCAGAGT	3'-RACE
APG	CCAGACTCGTGGCTGATGCA(G) ₁₆	5'-RACE
AP	CCAGACTCGTGGCTGATGCA	5'-RACE
<i>Lm</i> IL-10FL-F	ATGACTCCTCGGTCTCTCCTCC	Verify the full length
<i>Lm</i> IL-10FL-R	CGGTCACATTTGGATTAGGGTCA	Verify the full length
<i>Lm</i> IL-20LFL-F	CAGTGATGAAGATGCTGCTTGG	Verify the full length
<i>Lm</i> IL-20LFL-R	TGGGATTGATTCTGAGTGTCTTTG	Verify the full length
<i>Lm</i> IL-22FL-F	CTGAACCATGAAGCCCAACG	Verify the full length
<i>Lm</i> IL-22FL-R	AAAAGTTGTTGGTATAAAAAGGTGAT	Verify the full length
<i>Lm</i> IL-26FL-F	ACTTGAAGATGTTTCTCCTCCTCG	Verify the full length
<i>Lm</i> IL-26FL-R	GATGATGCCGGTGGAGGTGA	Verify the full length
<i>Lm</i> IL-10-qF	CTCCTTCAAACTCCGTTCCGC	qPCR
<i>Lm</i> IL-10-qR	CAGTCGGTGACATCACTCTTGAGC	qPCR
<i>Lm</i> IL-20L-qF	ATGAAGGACGTTCCAGGAGGGG	qPCR
<i>Lm</i> IL-20L-qR	CTTGATAAACTCAGCATGCAGGG	qPCR
<i>Lm</i> IL-22-qF	GTC AACACGGACCAGGACCAC	qPCR
<i>Lm</i> IL-22-qR	CCCATCTTGGCGAGCTTCT	qPCR

TABLE 1: Continued.

Primer name	Sequence (5'-3')	Purpose
LmIL-26-qF	GCACCAAATGTCCTGAGCGTAC	qPCR
LmIL-26-qR	GGGTTTAGAGGAAGAAACGCAGT	qPCR
Lmef-1 α -qF	AAGGGATGGAAGGTCGAGCGC	qPCR
Lmef-1 α -qR	CGTTCACGGGAGCAAAGGTCAC	qPCR

(ORF) encoding 187 amino acids (aa) (Figure 1(a)). The full-length cDNA of IL-20L of spotted sea bass was 1,075 bp, with 125 bp of 5'-UTR, 428 bp of 3'-UTR, and 528 bp of ORF encoding 176 aa (Figure 1(b)). The full-length cDNA of IL-22 of spotted sea bass was 1,093 bp in length, containing 136 bp of 5'-UTR, 381 bp of 3'-UTR, and 579 bp of ORF encoding 191 aa (Figure 1(c)). The cDNA length of IL-26 of spotted sea bass was 1,158 bp in length, including 9 bp of 5'-UTR, 615 bp of 3'-UTR, and 534 bp of ORF encoding 177 aa (Figure 1(d)). The polyadenylation signal (AATAAA) was identified in the 3'-UTR of the four genes of spotted sea bass (Figure 1). Multiple sequence alignment revealed that the obtained four ILs contained conserved cysteine residues and motifs of IL-10 family members (Figure 2).

3.2. Evolutionary Tree and Syntenic Analysis of IL-10 Family Members. Phylogenetic analysis showed that IL-10 family members formed four main branches, including IL-19/IL-20/IL-24 branch, IL-10 branch, IL-22 branch, and IL-26 branch (Figure 3). Among the IL-19/IL-20/IL-24 branch, IL-20L of spotted sea bass was well clustered with IL-19L of hybrid striped bass (*Morone saxatilis*) and IL-20L of large yellow croaker (*Larimichthys crocea*), supported with 99% bootstrap value. In the branch of IL-10, IL-10 of spotted sea bass was clustered together with IL-10 of European sea bass (*Dicentrarchus labrax*) and large yellow croaker, supported with 96% bootstrap value. IL-22 of spotted sea bass fell in the IL-22 branch and clustered with IL-22 of European sea bass, mandarin fish (*Siniperca chuatsi*), and largemouth bass (*Micropterus salmoides*). In the IL-26 branch, IL-26 of spotted sea bass was clustered with IL-26 of medaka (*Oryzias latipes*).

Further, gene synteny analysis found that the gene loci of the IL-10 family members of spotted sea bass were similar to other vertebrates. *IL-10* and *IL-20L* were found to exist in the same locus, and highly linked to *DYRK3* gene. Interestingly, we found that *IL-22* and *IL-26* located in the same locus, and always linked with *MDM1* gene (Figures 4(a) and 4(b)). Notably, the genomic organization of all four genes consisted of five exons and four introns (Figure 4(c)).

3.3. Tissue Expressions of IL-10 Family Members in Healthy Spotted Sea Bass. Results on the tissue distributions of IL-10 family members by using the qPCR quantification showed that the four genes were constitutively expressed in all selected tissues from spotted sea bass (Figure 5). However, their expressional patterns were different. IL-10 was highly

expressed in spleen, followed in gills and HK (Figure 5(a)). IL-20L, IL-22, and IL-26 were highly expressed in gills (Figure 5). In addition, IL-20L and IL-26 were moderate expressed in skin and intestine (Figures 5(b) and 5(d)). The four genes were lowest expressed in liver (Figure 5).

3.4. Expression Analysis of IL-10 Family Members in Spotted Sea Bass after *E. tarda* and LPS Challenge. It was found that the expressions of IL-10 family members of spotted sea bass were induced after *E. tarda* infection (Figure 6). The expressions of IL-10 and IL-20L in gills increased at 48 hr postinfection, the expression of IL-26 in HK increased significantly between 24 and 48 hr postinfection, the expression of IL-22 and IL-26 in intestine increased at 24 hr postinfection, and the expression of IL-22 in spleen increased at 24 hr postinfection.

Similarly, the expressions of the four members in the IL-10 family were also induced by LPS challenge in spotted sea bass (Figure 6). In gills, IL-10 was induced from 6 to 12 hr and IL-20L was upregulated at 12 hr post LPS challenge. In the HKs, IL-10 was upregulated from 6 to 12 hr and IL-22 was upregulated at 24 hr after LPS challenge. In the intestine, only IL-10 was upregulated from 6 to 24 hr after LPS challenge. In the spleen, IL-10, IL-20L, and IL-26 were upregulated at 6 hr and IL-22 was upregulated from 6 to 12 hr after LPS challenge.

4. Discussion

In the present study, four members (IL-10, IL-20L, IL-22, and IL-26) of IL-10 family were identified in spotted sea bass, providing basis for revealing the functions of these ILs in fish. Multiple sequence alignment showed that the four ILs had the signature motifs of IL-10 family (Figures 1 and 2). Also, several conserved cysteines that are important for the structural and functional stability of ILs were observed in IL-10, IL-20L, and IL-22 of spotted sea bass. However, conserved cysteines were found in IL-26 of spotted sea bass, being similar to that of grass carp IL-26 [15]. Further, in the constructed phylogenetic tree in the present study, IL-10, IL-22, and IL-26 of spotted sea bass were well clustered with their counterparts of fish and mammals (Figure 3). Fish IL-20L was clustered with IL-19, IL-20, and IL-24 of higher vertebrates, suggesting that fish IL-20L might share same ancestor gene with IL-19/20/24 of higher vertebrates. Similar results were also found in rainbow trout [37] and grass carp [13]. Further gene location provided some


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1      cacttgaagATGTTTCTCCTCCTCGTCAGGACGCTGTCTCATTCTGCTCATCAGCCTCGCCGGTGGGCTCGTCGCCGTGGCAACCGCC
1      M F L L L V R T S V L I L L I S L A G G L V A V A T A
91     GAGCGCGGCATCCTGCCGGCAGGAGATCCCCGCCGAGCTGATCCGAGACCTGTGGAGCCGGACACACAGCTGATCAACAAGCTGCCG
28     E R G I T C R Q E I P A E L I R D L W S R T T Q L I N K L P
181    AAAGAAGAAAATTCTCCGGCGAGTCAGACTGCTGCCAAAATTCGCACAAAATGTCTGAGCGTACGATTGGCTGGCTGGAGATCGCG
58     K E E K F S G R V R L L P K F C T K C P E R T I G W L E M R
271    CAACTGCTTGATGTTTATCAGAGGAGTGTGTTCCAGAGAGCTCGTCCAGGAGCTCCTCCCTCCACTACAATGACCTGTGTACCGA
88     Q L L D V Y Q R S V F S R E L V Q E L L P L H Y N D L L Y R
361    CTGCAACACACACTGCAGCACTGCGTTTCTCTCAAACCTCAAATGGTTCAAATCATCAAGAACTGGAGAGAAAAATTA AAAAG
118    L Q H T L Q H C V S S S K P S K W F K I I K K L E R K I K K
451    AGGAGGAGAGACGTAGGAGCGCTGAAGGCCGTCCGGAGAGTTCACCTTCATCCTCAGGTGGATTGATGAACTGGCACAGCACCGTCTG
148    R R R D V G A L K A V G E F T F I L R W I D E L A Q H H V L
541    TAActcatcatcctcatcctcatcatcatcatcagttatgtgatcatcacctccaccggcatcatcagcaaaattagggctcgTTTT
178    *
631    tagcagcacaacatgtacttttagacatttatttttaatttaattatctgagggaaaatctgaaatattttcaaaatgtttacctccagt
721    gtttgatgtgtttggcccaaaatttacactttaaaatctgttcaaacctgatctactttggcagaaaaaaatgtaaaaactgaaaatag
811    atctaatactctgatcttccacagctgtctgagcatctcaagaggcttgaatctatTTTTTTTaaagtattgattactgttttgat
901    gttttgataaatgtagctttatttcaaaaatagttattttcagaaaaatattttgtatttgagttacccttaagattccaggtgttaa
991    aagtacttgaacattaatatattattgttatatatattttctaacaatatgaaggaaaaatTTTTTatagaaatgtgtgtgtctgta
1081   tttacgtgaatttgttgatcagattcttagtga(aataaa)c tagatttgtatccaaaaaaaaaaaaaaaaaaaaaaaaaaa

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(d)

FIGURE 1: Nucleotide and predicted amino acid sequence of (a) *LmIL-10*, (b) *LmIL-20L*, (c) *LmIL-22*, and (d) *LmIL-26* in spotted sea bass. The start codon (ATG) and the stop codon (TAA) of each sequence are shown in bold red. The gray-shaded parts are signal peptides. Polyadenylation signals (AATAAA) were boxed.

information for us to understand the evolutions of the members of IL-10 family in fish. Fish *IL-10* loci contained *IL-10* and *IL-20L*, while mammalian *IL-10* loci had *IL-10*, *IL-19*, *IL-20*, and *IL-24* (Figure 4). Fish seems to lack *IL-19* and *IL-24* [38]. It was, thus, speculated that the divergence of *IL-19* and *IL-20* might occur during the evolutionary process after fish appearance [13, 33]. Moreover, the gene organization also reflects the evolutionary conservation of the four *IL-10* family members in spotted sea bass. They all have a five exons/four introns structure, as observed for the *IL-10* family coding region in mammals and other fish species [19, 39, 40]. These results confirmed that the genes we cloned in spotted sea bass were exactly the members of *IL-10* family.

We found that the *IL-10* family members of spotted sea bass were prevalent in all tested tissues, but they were expressed at different levels (Figure 5). *IL-10* of spotted sea bass was highly expressed in spleen and gills, similar to that of *IL-10* of goldfish (*Carassius auratus* L.) [41], swamp eel (*Monopterus albus*) [19], and *Oreochromis niloticus* [9], while other three *ILs* were highly expressed in gills. Also, the expression levels of *IL-20L*, *IL-22*, and *IL-26* were relative lower than that of *IL-10*. *IL-20L* of grass carp [13] and snakehead (*C. argus*) [12] was highly expressed in the HK and liver, while *IL-20L* of spotted sea bass was lowest expressed in liver. These results indicated that the expressions of *IL-10* family members of fish might be species specific and tissue specific. Except for *IL-20L*, the expression of the other members was also lowest in the liver. Previous studies have shown that *IL-10* family is closely related to liver-related diseases, but whether it is the same in fish that needs further investigation [42–45]. Gills are important mucosal organ of fish, which are able to mount immune response to protect the fish from pathogens [27, 46]. Highly expression of *ILs* might involve in the regulation of mucosal immunity of spotted

sea bass. It had been found that zebrafish *IL-10* was essential for maintaining gills homeostasis [27].

E. tarda causes hemorrhagic septicemia in fish, leading to mass mortality [47]. Following *E. tarda* infection, the *IL-10* family members of spotted sea bass were induced in several immune-related organs (Figure 6). Similarly, *IL-10* of Nile tilapia was dramatically increased in HK and spleen following *Streptococcus agalactiae* infection [9]. *IL-20L* of rainbow trout was upregulated after *Yersinia ruckeri* infection [18], *IL-20L* of grass carp was increased in HK after *Flavobacterium columnare* challenge [13], and *IL-20L* of snakehead was induced in the HK and spleen after *Aeromonas schubertii* and *Nocardia seriolae* stimulation [12]. It had been found that fish *IL-22* could be upregulated by several bacterial pathogens, such as *Aeromonas salmonicida* [48] and *Edwardsiella ictaluri* [49]. *IL-26* is thought to be a novel antimicrobial peptide, and the T cells that can produce *IL-26* take part in the immune response against enterotoxin produced by *Staphylococcus aureus* [50]. It had been found that *IL-10* could promote the immunoglobulin (Ig) M antibody production in Nile tilapia [9]. These results indicated that the *IL-10* family members involve in the host immunity against bacterial pathogens.

LPS is the main component of the outer membrane of Gram-negative bacteria [51]. We found that the *IL-10* family members of spotted sea bass were also upregulated by LPS in the immune-related organs (Figure 6). Our results were in line with previous studies [9, 18, 52]. LPS could induce the inflammatory responses in fish [53]. These results suggested that fish *IL-10* family members involve in the immune regulation of inflammatory responses. In addition, fish *IL-10* family members also involve in the immune response to other pathogens. Mandarin fish *IL-10* was significantly increased in spleen after infectious spleen and kidney necrosis virus (ISKNV) infection [54].

IL-10 *D. labrax* : MTPRSLLSILVY--LSFFITVWCSPMCNNOGCRVEGFGMQLRADFTEHQDYFYEAND-DIDAAIDQTVEDTLKTPFACHAINSLDFYISTVLFPTAMAG: 101
 IL-10 *L. crocea* : MTPRSLLCALVL--LSFFITVWTSVPCVKNKCRVEGDFVRLKRLNVAERDYFYEAND-DIDTALDQSVVEETFKTPFACHAINSLDFYIATVLFPGALAG: 101
 IL-10 *L. maculatus* : MTPRSLLSIVLVL--SSFFITVCCCLPTCNKCRVEGFGVRLKRLREYDSQRDYFYEAND-NIDTALDQSVVEDSFKTPFACHAINSLDFYIDTILVLFALAG: 101
 IL-10 *T. rubripes* : MTPGS-LSVLVL--LCCACTVWCAALCNKRCOSVEGFGARLKMREYNSQRDYFYEAND-DIDIVLDQSVVDTFKTPFACHLLDGMRLRFYDSVLFALAT: 100
 IL-10 *C. carpio* : MIFGTGVSALVM-LLLTDSAQCRVDCKSDGCTVEGFGVRLKRLRSAYREIQRVYFYSND-DIEP-LNENVQONINSPYGQHVINEELRFYETILVFTAVQK: 101
 IL-10 *C. idella* : MIFSRVFSALVM-LLLSESAQCKVDQDQCSVEGFGVRLKRLRSAYREIQRVYFYSND-DIEP-LNENVQONINSPYGQHVINEELRFYETILVFTAVQK: 101
 IL-10 *D. rerio* : MIFSGTIVSALVT-LLLCDCAQSRVVECKTDQCSVEGFGVRLKRLRSAYREIQRVYFYSND-DIEP-LNEDIKHNINSPYGQHVINEELRFYETILVFTAVQK: 101
 IL-10 *O. mykiss* : MSPSCLLSLLAAALQCEHAQCRVPCSDRCOSVEGFGVRLKRLRTAFSTIRDYFYEAND-EIETS-LDEGLLHLLKSPVGGHAMDLSLRFYIDTILVFTAMNN: 103
 IL-10 *H. sapiens* : --MHSSALCCIVLLTGVRAASPQGTQSENSQTHPGNLDLDAFASRVKTFQMKD-QDNLCKESLLEDFKGLGCOALSELRQYIEEYMPQAEK: 100
 IL-10 *M. musculus* : --MPGSA-LCCVLLLTGMRISRGQYSREDNQTHPVGQSHMLLRTAFASQVKTFFQTKD-QDNI-LTDSLMDQDFKGLGCOALSELRQYIEEYMPQAEK: 100
 IL-10 *X. laevis* : --MK-FCVLLTFFFT-CKTVRCQSDAEGSQRVNIIFAKKELRATFQKKNFQMKQNNETV-LQNDLQEFKGNMGQRSETRFYEDVLRQAN: 98
 IL-10 *G. gallus* : --MOTCCQALVLLAAACTLPAHCL----EPTGLHSELLARLLELRVKFEETKDYFCRDEENIQLSSELEDEFKGTFGQOSVSELRFYTDEVLRFRAMQ: 97

IL-10 *D. labrax* : VTEDTKDLKPHVESIQIFDQKSDVTRDRHYFKCKH-HFDINTNSIVYTOJESKGLYKAMGELGLFNLYETVYASQQRNHAASV: 187
 IL-10 *L. crocea* : VTEDTKSMKPHVESIQIFDQKNDVTDQRHYFKCKH-QFDINTNSIVYTOJESKGLYKAMGELGLFNLYETVYASQQRNHNH: 184
 IL-10 *L. maculatus* : VTEDIRSLKPHVESIQIFDEKSDVTDQRNWFCKK-EFDIKTNSIVYTOJESRGLYKAMGELGLFNLYETVYAFKRRHNHVPV: 187
 IL-10 *T. rubripes* : VTAETRNLLKPHVESIQIFDQKIEVTVNKHVYFACKH-RFDINVNSIVYTKJEDKGLYKAMGELGLFNLYEINYLAKRRRNVA: 183
 IL-10 *C. carpio* : NHL---RSTTPIDSTIGNIFQDKRDTRKRNWFSCON-PFEIASKNSYEKKEKGVSKAMGELDLFKYIEQVYASQVRVKHL: 180
 IL-10 *C. idella* : NHL---HPKTPIDSTIGSIFQDKRDTRKRVKWFSCN-PFEFATKNSYEKKEKGVYKAMGELDLFKYIEQVYASQVRKHK: 179
 IL-10 *D. rerio* : NPLK--HSTTPIDSTIGNIFQEKRDTRKRVKWFSCN-PFEVNSKNSYEKKEKGVYKAMGELDLFRYIEQVYASQVRKH: 180
 IL-10 *O. mykiss* : RTQNNDFKSPIDSTIGNIFHEKKEVQRNWFSCCK-PFDINEFISSEYEKJODKGLYKAMGELDLFNLYEIEVYVSKRRKH: 184
 IL-10 *H. sapiens* : --QDPDIKAHWNSGENLKTILRLRRRHRRLPCENKSKAEQVKNANKQEKGLYKAMSEFDFINLYEAVYMTKIRN: 178
 IL-10 *M. musculus* : --HGPEIKHEHNSGEKLLKTRMLRRRHRRLPCENKSKAEQVKSDFNKJODQGVYKAMNFFDFINCEAVYMTKIKNS: 178
 IL-10 *X. laevis* : --HYKMNWVLDQKLLDKHTLRRRHRRLPCRKSKAKELKQVYKWRQGLYKAMGEPDIDYIEEYVLSRKK: 172
 IL-10 *G. gallus* : --TSTSHQSSIGDGNMLLGGKATRRRHRRTCEKRSKAKQIKETIEKMDENGLYKAMGEPDIFINLYEYVLLMRRK: 175

(a)

IL-24 *H. sapiens* :MNFQRLQSLWTLASRPFCPLLATASOM--QMVVLPCEGFTLLWSQVSGAQGEFFHFGPQVKGQVPOKWEAWAV: 77
 IL-24 *M. musculus* :MLTEPAQLFVHKKNQPPSHSLRHFRTLALALSSQTMSWGLQILPCLSLILLNQVQVPLEGQEFRFGSCVTVVLPPEWEAWTV: 90
 IL-19 *H. sapiens* :MKLQCQSLWLTGTLILCSVDNHG----LRRCLIS-TDMHHEESQET: 44
 IL-19 *B. taurus* :MKAPCVSLCLGAGFLCSVHARG----LRRCLIS--NLHRVEESRCH: 44
 IL-19 *M. musculus* :MKTQCASTWLGMTILCSVHIYS----LRRCLIS--VDMRLLEKSHCH: 44
 IL-20 *G. gallus* :MLGSRVLLCICSMTCCLTMLPAAGNTLHFHGPCHISMSBRAGATAI: 49
 IL-20 *H. sapiens* :MKASSLAFSLSAFYLLWTPSTGLKTLNLGSCVIA-TNLQERNGISEI: 49
 IL-20 *M. musculus* :MKGFGLAFGFSVAGVLLWTPLTGLKTLHLGSCVIT-ANLQATQKEISEI: 49
 IL-19L *D. rerio* :FICIVLSGLWDAQGRRLHLGSCVKN--HTHERHHQYV: 40
 IL-20L *C. idella* :MKDLFIYSVFCVILCGLWKAHGRRLHLGSCVKN--HTHERHHQYH: 49
 IL-19L *M. saxatilis* :MKMILS-RSICLVLLSCLSELIESRTLHLNSCSVN--VHTHERKYSTI: 48
 IL-20L *L. crocea* :MMLRELCONTREKSPSVHYKSLRHGDEAHLSDQSSSEHTQTHSPTRHQEM: 100
 IL-20L *L. maculatus* :MKMLLG-HSICLCLLSRLGELVESRALHLSDCSVN--VHTQERKYSTI: 100
 IL-20L *T. rubripes* :MKMLLG-CSICLCLLSCLRELVESRTLHLSDCSVN--VHTHERKHSATI: 48
 IL-19L *O. mykiss* :MKTLLSCLFLFLCCLTKPARSQTLLNSCSVN--VNLLEEARKHYSH: 49
 IL-20L *X. laevis* :MKLLLSPTIPRLLFLACLSCGGLGHGHLGTCVSTVHTHERKHYSH: 49
MVALSAVSTVVCILMMKIKIESSGHHCPVLS--DIQEFKHYHESV: 46

IL-24 *H. sapiens* : KDTQAQDNITSARLQ-QEVLQNSDAESVYLVHTIEYIKTVEKNYHNRTVEVRTLKSFSLANNVILVQSQVOPSQENMFISIRD SAHRFLFRRA: 178
 IL-24 *M. musculus* : KNTIQDQDITSLRLEK-PQVBRNLSGAESVYLAHSIKYVYVYKNSYHNSIAKFKVLRSFSLANNVIVMSQVOPSKDMSMLPISIAHRFLFRRA: 191
 IL-19 *H. sapiens* : KTAQAQDTPFNITLSTLETQIKPLDVCVTKNKAIVYIDRVFKDHO--EPNPKILRKISLANS-LVMOVTEQQVQRHOR/EATNATRIHDNV: 144
 IL-19 *B. taurus* : KTAQAQDTPFNITLSTLETQIKPLDVCVTKNKAIVYIDRVFKDHO--ELNPQIMRKRISLANS-LVMOVTEQQVQRHOR/EATNATRIHDNV: 142
 IL-19 *M. musculus* : KTAQAQDTPFNITLSTLETQIKPLDVCVTKNKAIVYIDRVFKDHO--ERSLEVLRRISLANS-LVMOVTEQQVQRHOR/EATNATRIHDNV: 143
 IL-20L *G. gallus* : KTNQAQDPIRTSLSHPHSLHRVQPSDKCIVHKVFNLYDKVFKHCQ--TENSYNIRKISLANS-LSIKKLEQVCHENKLOGEPTERFKQLVNV: 149
 IL-20 *H. sapiens* : RGSQAQDGNIDIRLRTTESLQDKTPANRCOLLRHRLYIDRVFKYQ--TPDHYTLRKISLANS-LTIKIDRLCHAHTCHQGEAMKYSQLSH: 149
 IL-20 *M. musculus* : RGSQAQDGNIDIRLRTTESLQDKSLDRCFLLRHRLYIDRVFKYQ--TPDHYTLRKISLANS-LTIKIDRLCHAHTCHQGEAMKYSQLSH: 149
 IL-19L *D. rerio* : RQGISGDDHKGRLR-KDVVNSQATESCFLSQHLHYIDRVISYV--SSHSHRRTSVLANS-LSHSIDRVHANAHQEGNTRLOKLSQTA: 139
 IL-20L *C. idella* : RQGISGDDHKGRLR-KDVVNSQATESCFLROHLHYIDRVISYV--SSOSLHRTSVLANS-LSHSIDRVHANAHQEGSDARLKLTSQTTY: 148
 IL-19L *M. saxatilis* : RSNIAAGDVTIGIKFK-KSLITDQDGTQFLRLVQYERVRSNYA--SAQPQDQCSALANAIVSIRDIHKCH--CHQETQRTVDSHAE: 143
 IL-20L *L. crocea* : RSNIAAGDVEVAKVS-KSLTKNQDQGMCFLLRLVRYERVRSNYA--SAQPQDQCSALANAIVSIRDIHKCH--CHQETQRTVDSHAE: 195
 IL-20L *L. maculatus* : RSNIAAGDQVIGIKFD-KSLTKDQEGQCFLLRLVRYERVRSNYA--SQPEQDQCSALANSIVSIRDIHKCH--CHQETQRRIDSHAE: 143
 IL-20L *T. rubripes* : RSNIAITGDDEIGKFD-KSLIEDQDGORCFLLRLVRYERVRSYV--SQPDERILSALANTITIRDIHKCH--CHQETQRRVDAHQAE: 144
 IL-19L *O. mykiss* : RSNIAAGDQVIGIKFD-KSLTKDQEGQCFLLRLVRYERVRSYV--SQPDERILSALANSITIRDIHKCH--CHQETQRRKMSDAQE: 144
 IL-20L *X. laevis* : RSNIAAGDQVIGIKFD-KSLTKDQEGQCFLLRLVRYERVRSYV--SQPDERILSALANSITIRDIHKCH--CHQETQRRKMSDAQE: 144
 IL-20L *X. laevis* : RSNIAAGDQVIGIKFD-KSLTKDQEGQCFLLRLVRYERVRSYV--SQPDERILSALANSITIRDIHKCH--CHQETQRRKMSDAQE: 144

IL-24 *H. sapiens* : KQDVEALTRAGVILITWIKFYKL-----: 207
 IL-24 *M. musculus* : KQDTEVALTRAFGVILITWIKFYHL-----: 220
 IL-19 *H. sapiens* : DQLEVHAIAIKSGLDVFALWIKNHNEMVSSA----: 177
 IL-19 *B. taurus* : DQLEVRSIAVKSGLDVFALWIKHHRGTSAASR--: 177
 IL-19 *M. musculus* : NQLEVSSALIKSGLDILAWIDRNHLETAA----: 176
 IL-20L *G. gallus* : EGNVTSAMKSGLDILQWIKSP-----: 176
 IL-20 *H. sapiens* : EKLEPQAVVKAGLDILQWIKETE-----: 176
 IL-20 *M. musculus* : IELELQAVVKAGLDILQWIKEML-----: 176
 IL-19L *D. rerio* : EKLDQAAGTVKAGLDILQWIKSFQQ-----: 168
 IL-20L *C. idella* : DKLDQAAGAVKAGLDILQWIKSFQHH-----: 177
 IL-19L *M. saxatilis* : IKLEIKQAQKAGLDILQWIKGLTHDQHTHT----: 176
 IL-20L *L. crocea* : IRLQEQQAQKAGLDILQWIKGLGQNSLR-----: 227
 IL-20L *L. maculatus* : IKLEIQQAQKAGLDILQWIKGLSGLKTOP-----: 175
 IL-20L *T. rubripes* : NQLEIGKARKAGLDILQWIKDSEKQSPN-----: 176
 IL-19L *O. mykiss* : DKLEIQQAQKAGLDILQWIKELKHNSTHTDR: 181
 IL-20L *X. laevis* : YQLETEAIIKAGLDILQWIKKNYQG-----: 174

(b)

FIGURE 2: Continued.

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IL-22 M. salmoides : ----- MKLVAAEVVSLRFP ---AM V L P LLIGWVQAATLP VDRPLSQPLRNPETYEAVRDKVSKHQRV : 63
IL-22 S. chuatsi : ----- MKLFAAKVVSFLRPA ---AM V L P LLIGWAEQAALP VDRPLSQPLRNPETQYAVRQVSKHQAQSV : 65
IL-22 L. maculatus : ----- MKPNAAAVVSFLRPAATA M V L P LLIGWAEAAASH VNRLSQPLQDDTYAVRQVSKHQAQSL : 67
IL-22 D. labrax : ----- MKL ---ATL VSVLCPAA ---AM V L P LLIGCGEQVAALP VDOPLSKPLRDPETQYAVRQVSKHQAQ : 61
IL-22 O. mykiss : ----- MKFSTVQLVA ----- AV V M S G C L L --- RESVAHS IHRPLSAPLHSAADTDMVQVQAQHAQ : 54
IL-22 C. carpio : ----- MKF T T F A M C C C C F L R G Q A M H L S R P R P K P L D S S A T W N D L F M T K H A Q : 48
IL-22 C. idella : ----- MKF T T L A M C C W C F L C G Q A M H L M R P R P K P L D S P V T W N N L Y V M T E H A Q : 48
IL-22 D. rerio : MDGYAKGEKTTT VYRHDIKAPEPQDALQVSTSRNNGVHKRTDTRIHSSCT DMKCF T I A L C S - C F L S G ----- CARPTLDSSATWNDLAAITD TAR : 93
IL-22 H. sapiens : ----- MAALQKVSVSFLMGLTATSC ----- L L L A L V O G G A A P I S S H --- C R L D K S M F Q Q P Y I T N R T F M I A K E A S : 64
IL-22 M. musculus : ----- MAVLQKSMFSFLMGLTAA S C ----- L L L A L W A Q E A N A L P V N T R -- C K L E V S N F Q Q P Y I V N R T F M I A K E A S : 64
IL-22 G. gallus : ----- MATLHT - L T R S F S G W V F C C C C F P L L T S P P P K G T G V S N A H Q A C R L R K I N F Q Q P Y I R N R T Y T I A E M A R : 70
IL-22 X. laevis : ----- MAALFLQMRKFAAFWILFCCCYLSD I L C A P D P Q E N H P V S K Q H - L C T I R K T F F L Q T F M K K N I F A I A E Q A R : 70

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IL-22 M. salmoides : QTED S S T R L P R V N T D Q D H L K - I C C H A N I L D Y L N N I L P H R --- D N Q H P K H R K T D T T V S E D I Q T G G C N V T H Y D H H H A V E F I R K L A T M G --- G E R G : 157
IL-22 S. chuatsi : QTED S S T R L P R V N T D Q D H L K - I C C H A N I L D Y L N N I L P H R --- D N Q H P K H R K T D T T V S E D I Q T G G C N V T H Y D H H H A V E F I R K L T K M G --- G E R G : 159
IL-22 L. maculatus : QTED S S T R L P R V N T D Q D H L K - I C C H A N I L D Y L N N I L P H R --- D N T H P S H R K T D T T V S E D I Q T G G C N V T H Y D H H H A V E F I R K L A K M G --- G E R G : 161
IL-22 D. labrax : QTDDGSSARL P R V K A D Q D H L K - I C C H A N I L D Y L N N I L P H R --- D N A H P S H R K T D T T V S E D I Q T H G C N V T H Y D H H H A V E F I R K L A R M G --- G E Q G : 155
IL-22 O. mykiss : S S D T T D T K L P D I D T K N H R D - I C C H A N I L D Y L S N I I T T K E Q K H H P K P A K E D I A V S R D L K E H G C A I K H Y N D H H S I A F I K K L S E M E --- E G K G : 151
IL-22 C. carpio : M E D T H E T R L P V L S E V M L K E E S S C C N A M L N Y L K H I L H A D E H V K K Y P N R F R S D Q I A H I K P H - C E T S D F V D H V R V K E F E K N Y K T A S E K F - E K T : 148
IL-22 C. idella : K E D K H E T R L P V F S E V M L K E E G T C C H N T M L D Y L K H I L H A D D H V K - Y P O R F R S D H L A Q D I K P Y - C S N S - - A E H A Q V K K Y D R Y S A Y E R K S T L E A : 146
IL-22 D. rerio : N E D - H E T R L P Y F S H D M L Q E E G S C C H N A R I L K Y Y N H V L S E D H T M K Y P N R N R E G H V E Q E I Q N H - G K H D - Y S S H P L V K Q F R N Y H A S A I M D L - A A A : 191
IL-22 H. sapiens : L A D N T D V R L G E K L F H G V S M S E R Q Y L M K Q V L N F T L E E V L P Q S - - R F Q P Y Q E V P F A I L S N R S T C H I E G D D L H I Q R N V Q K L I D T V K K L G --- E S G : 159
IL-22 M. musculus : L A D N T D V R L G E K L F R G V S A K D Q Y L M K Q V L N F T L E D V L P Q S - - R F Q P Y Q E V P F A I L S N Q S S C H I S G D D Q N I Q K N V R R L I E T V K K L G --- E S G : 159
IL-22 G. gallus : L S D Q T D N R L G Q Q T Y V N I R E N N R Q Y L M K R I T E I I V K D I L L T E A - K E R Y P Y A E D A Q F A S L T S E S R C K Y S G N R E H I E K N L E E M S K M K E L G --- E N G : 165
IL-22 X. laevis : I M D K T A N K I F G S Y L F F G V K E K D H V L M K N V N S F V E N V H - E S - S K K Y P H D N A I A F F V N I E K D L A G C K S E E G Q - I Q R N V E Q M I N K I K M M G --- P D G : 163

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IL-22 M. salmoides : I N K A V G E I D I L F T Y I Q D F L Q L K N S T A T L A A A H ----- : 190
IL-22 S. chuatsi : I N K A V G E I D I L F T Y I Q D F L Q P K N S T A - - A A A H ----- : 190
IL-22 L. maculatus : L N K A V G E I D I L F T Y I Q D F L Q P R N A S T - - - A A F ----- : 191
IL-22 D. labrax : I N K A V G E I D I L F T Y I Q D F L H P R N S T A A A T A N T S A V S T E Q : 195
IL-22 O. mykiss : I K K A T G E I D I L F T F L K D F V H A ----- : 173
IL-22 C. carpio : R N K A V G E T I L F H Y L F E S S N P R I ----- : 171
IL-22 C. idella : R N K A V G E T I L F H Y L F E S S A R M ----- : 169
IL-22 D. rerio : R N K A I G E T I L F H Y L F E S S T P K - - - - - : 213
IL-22 H. sapiens : E H K A I G E L D L L F M S R N A C I ----- : 179
IL-22 M. musculus : E H K A I G E L D L L F M S R N A C V ----- : 179
IL-22 G. gallus : K N K A I G E L D L L F Y H E N A C T D A P K K G G N K K K N - - - - - : 197
IL-22 X. laevis : K N K A I G E L D L L A H I R K C T L Q R M K P H A N K - - - - - : 192

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(c)

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IL-26 S. scrofa : M Q V N C I L R G L F V I T L S L A I A R R K Q S S F A E S C Y P R G T L S Q A V D T L Y V K A A R K A T I P E D R I K N - - T Q L L K K K T K L F M - K N C R F Q E Q L S F M E D V G R L Q L Q : 100
IL-26 B. indicus : M W V S C I L R C G L F V I T L S L A I A K H K Q S S F A E R C Y P R G T L S Q A V D T L Y V K A A S R A T I P E D R I K N - - T R L L K K K T K L F M - K N C R F Q E Q L S F M E D V G L Q L Q L Q : 100
IL-26 H. sapiens : M L V N F I L R C G L F V I T L S L A I A K H K Q S S F T K S C Y P R G T L S Q A V A D L Y I K A A W K A T I P E D R I K N - - T R L L K K K T K Q F M - K N C Q F Q E Q L S F M E D V G Q L Q L Q : 100
IL-26 G. gallus : M K V Y S I F R S G H L V L L C L F T V E G K K S P T G K H T C R K G L L S Q V T E N L Y T K A S S K S S V P K L I K N - - T R L L K K T T M L F M - T N C N V R D Q L S F Y M K N V S H L G M E : 100
IL-26 C. idella : ----- M R I V T L A L C A L L C C S Q G H K E E Q L R N H I Q L P L T K D M I N T S E L Q K P D R D R N P F - - H R I L G K L - K K C Y K - K L N V A D L K R L E I V E H V L K M M K N : 92
IL-26 L. rohita : ----- M R I V T L A L C A A L C C S Q G H K Q E E Q L S R H I P L T M K D M E T S E H I Q S L P R D E P F - - Q I L G L S L R C S R - K L N V A D F K R L E I V E H V Q K L W K N : 93
IL-26 D. rerio : ----- M R I L I P F T L C A L L W S E G H K Q E E Q L K R E I R L P M D R E L M S M S O D I H K S L P R N K P F - - H R I L G K L - K K C K - E L N V P D F K R V E I V D E H V E K M M D - : 90
IL-26 O. melastigma : M S P I I L R T L C L S L V S F I S G A V I T T A R L T L N C S E R V H P K L Q D L W N W T K L K E L P - E D K S P - - V R L V K F C V S C S K R V I G W M E R I R V D V V Q A H V S S K A L E : 101
IL-26 L. maculatus : M F L L L V R T S V L T L I S L A G L V A V A T A E R G I T C R Q E I P A E L H R D L W R S T T Q I N K L P K E E K F S G R V L L P K F C T K C P E R T I G W L E M R Q L D V V Q R S V S R E L V Q : 104
IL-26 X. laevis : - - M K F C N S L L S L V Y L T S L Y V F T C E T I K F H P K H C Q R A Q L E K N T E D L Y N K T V S F K R L F K C N I T D - - I Q L L T K Q L K E D F M A Q K N C N L R N L S F I N T F L E N T L V K : 100

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IL-26 S. scrofa : A - - C K E T D V E D F H S L R Q L S R C I S C A S S A R - - - E M K S T R K R I F Y G I G N K - G I Y K A I S E L D I L L S W I K O F L E S F K - - - - - : 171
IL-26 B. indicus : V - - C K E M H V E D F H S L R Q L S R C I S C A S S A R - - - E M K S T R K R T F Y E I G K K - G I Y K A I S E L D I L L S W I K O F L E S I K - - - - - : 171
IL-26 H. sapiens : G - - C K K I R V E D F H S L R Q L S H C I S C A S S A R - - - E M K S T R K R I F Y R I G N K - G I Y K A I S E L D I L L S W I K K L E S S Q - - - - - : 171
IL-26 G. gallus : - - - S E K L F V I S A F R V L Q E N M N A C L P C A P S T R - - - L T S A V K N K K T F L K L G E K - G V Y K A I N E L D I L L P W I Q A Y I Q T I V - - - - - : 170
IL-26 C. idella : D T H S T K K F M D S F M R L K N V M E I C E P K G Q K T L S I C A R E N I K T I E D T L M T D E P G - V H I K A I R F R S V L W I S L A I D R S R T H E K I Q : 174
IL-26 L. rohita : N T H Q L K M T D S F A R L K D M V E I C E T K G Q T L S L C A R E N I K T I E D T F K T L O P K - G L F K A Q S F R N V L W I S S A I D K R R T H E I H - : 174
IL-26 D. rerio : - - - E L P T Q I D Y F K R L K G I M Q N C A T E G K P T Q S R C A R E K K K F E Q T L M K L Q P D - C K T K A I S E F H S V L W I S S G L D R R K Y K K I H : 169
IL-26 O. melastigma : K - - L L H V H I N D V L H R L Q N V L Q N C V S S E P S K - - - F A E E I Q E I G T K I K K H H - - G A M K A A S E F T V L G W I S E L K - - - - - : 167
IL-26 L. maculatus : E - - L L P L H I N D L Y R L Q H T L Q H C V S S S K P S K - - - W F K I K K I E R K I K R R R D V C A L K A V G E F T F I L R W I D E L A Q H H V L - - - - - : 175
IL-26 X. laevis : E K - A K K F K I I D K L M V I Q D L L L H C K K S H C D Q E - T E S K S F R E L K K K T C Q I H G K R V L W I K A I S E M D I L V E W I Q E Y I E E M L - - - - - : 175

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(d)

FIGURE 2: Multiple sequence alignment analysis of (a) *LmIL-10*, (b) *LmIL-20L*, (c) *LmIL-22*, and (d) *LmIL-26*. The conserved cysteine residues in the mature peptide are marked by black triangles. Red boxes indicate IL-10 family signature motifs.

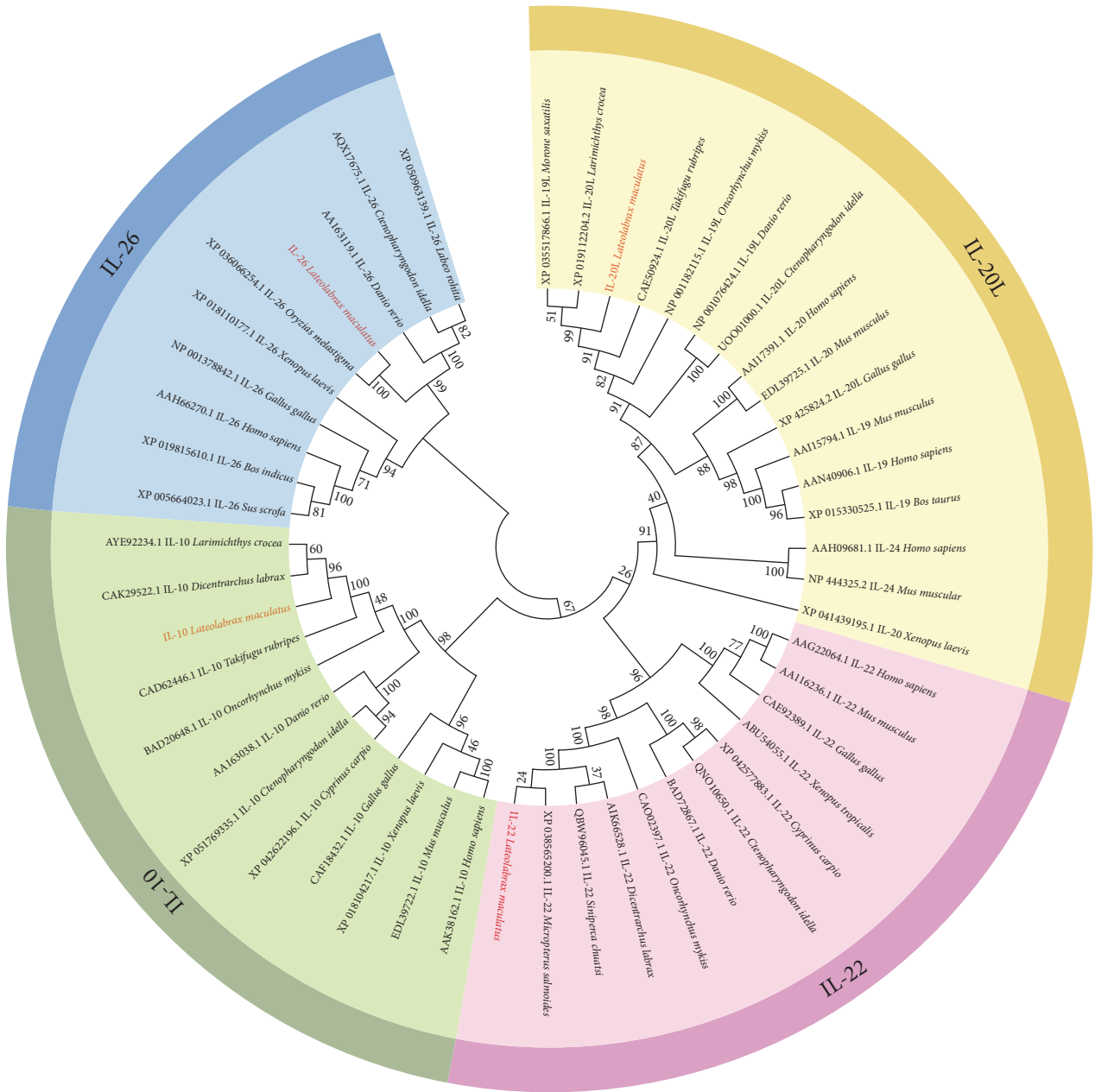


FIGURE 3: Based on amino acid sequences of the vertebrate IL-10 family homologues, phylogenetic trees were constructed by neighbor-joining method using MEGA 5.1 software.

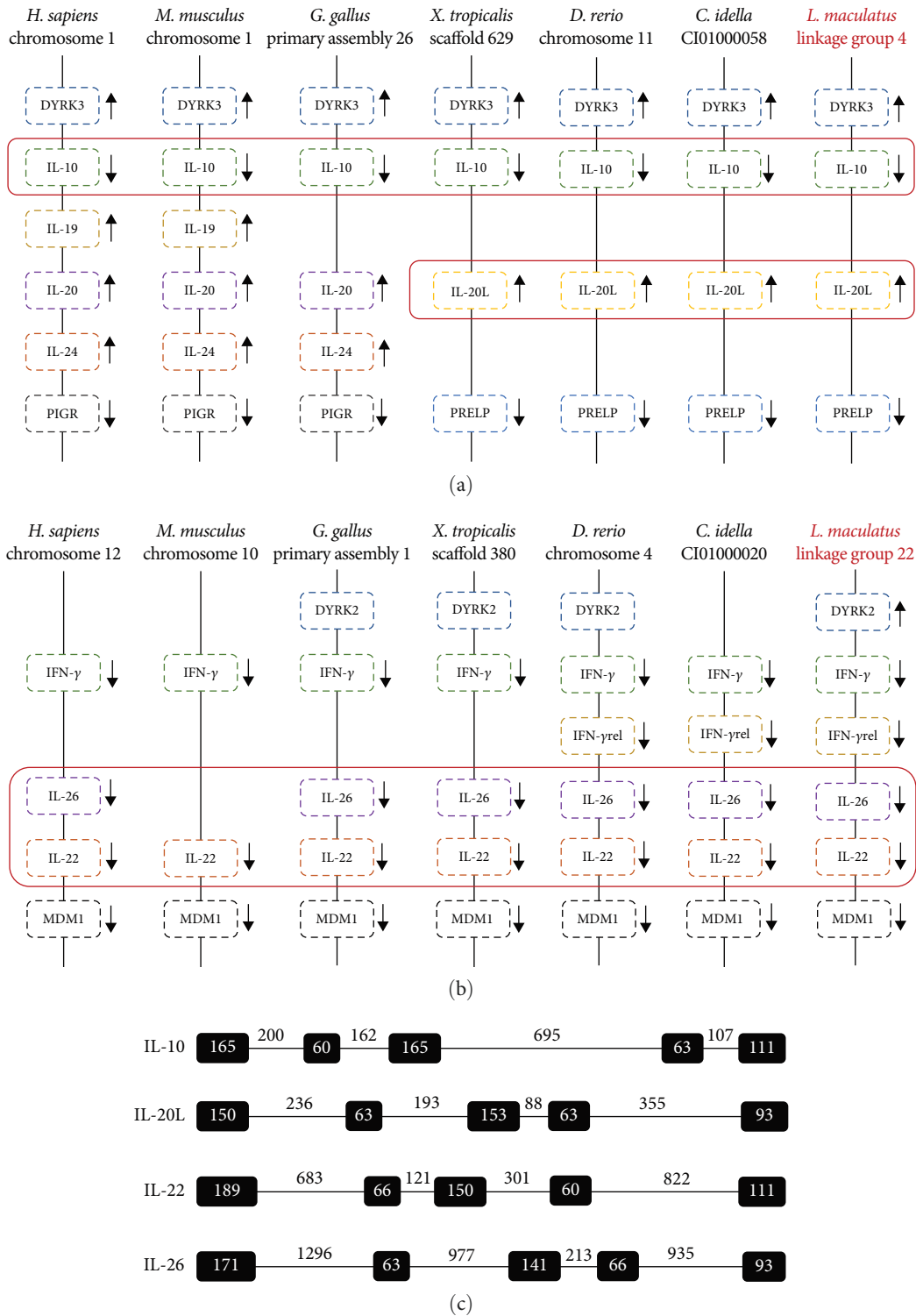


FIGURE 4: (a) Gene synteny analysis of *IL-10* and *IL-20L* (or *IL-19*, *IL-20*, and *IL-24*) genes in human (*Homo sapiens*), mouse (*Mus musculus*), chicken (*Gallus gallus*), tropical clawed frog (*Xenopus tropicalis*), zebrafish (*Danio rerio*), grass carp (*Ctenopharyngodon idella*), and spotted sea bass (*Lateolabrax maculatus*). (b) Gene synteny analysis of *IL-22* and *IL-26* genes in *H. sapiens*, *M. musculus*, *G. gallus*, *X. tropicalis*, *D. rerio*, *C. idella*, and *L. maculatus*. (c) The gene organizations of *IL-10*, *IL-20L*, *IL-22*, and *IL-26* in spotted sea bass. The blank boxes indicate coding exons, and the numbers indicate the size (bp) of exons and introns.

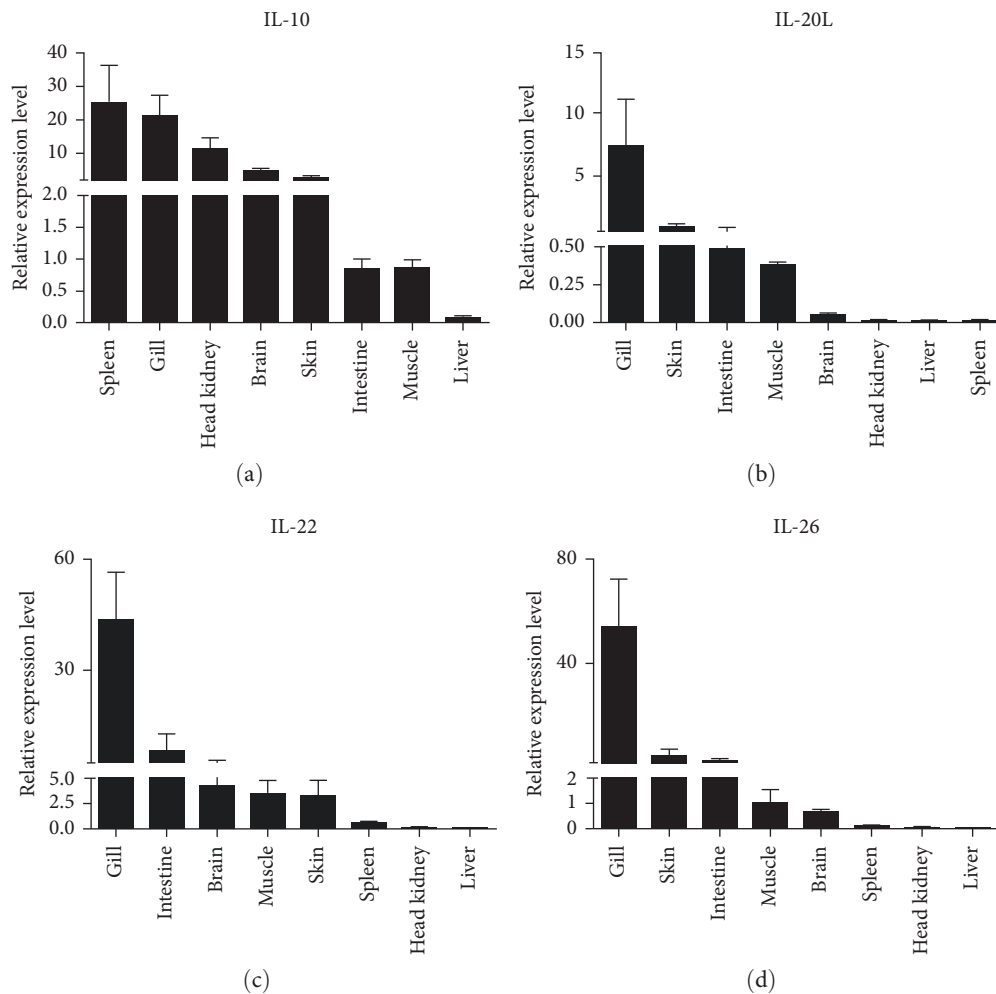


FIGURE 5: Expression of (a) *LmIL-10*, (b) *LmIL-20L*, (c) *LmIL-22*, and (d) *LmIL-26* in the tissues of spotted sea bass. Transcripts of IL-10 family cytokines were examined by qPCR in eight healthy tissues of spotted sea bass. The *Ef1 α* gene was used as the internal reference gene. The results are shown as mean \pm SEM ($N = 4$).

Also, frog (*Xenopus tropicalis*) IL-26 could be induced in spleen after Poly (I:C) stimulation [55]. These results confirmed that the IL-10 family members involve in immune response to pathogens invasion.

The studies on the IL-10 family cytokines in fish, especially their biological functions, are limited. Fish IL-10 has typical anti-inflammatory function similar to mammalian IL-10, and is a potential target for teleosts to resist microbial invasion and viral infection [10, 41]. Besides, it had been found that mandarin fish IL-10 could induce the proinflammatory cytokines, such as IL-6, IL-1 β , IL-8, and tumor necrosis factor (TNF)- α , indicating the anti-inflammatory role of fish [54]. Furthermore, IL-10 of Nile tilapia could promote the IgM antibody production in B cells [9]. Fish IL-20L also plays a vital role in the inflammatory response involving in regulating the immune response and promoting the proliferation of HK leukocytes [12, 13]. Fish IL-22 had

roles in maintaining intestinal homeostasis and regulating the expression of antimicrobial peptides in the mucosa-associated tissues [49, 56–59]. Fish IL-26 might be proinflammatory cytokine and involved in regulating T-cell-related immune response [15, 55]. The roles and functional network of fish IL-10 family members need further investigation. Our results provide a good starting point for further studies on the biological functions of the IL-10 family and their expression patterns after viral infection in spotted sea bass.

In conclusion, four members of the IL-10 family were identified from spotted sea bass and their expression patterns in normal tissues and immune-related tissues following *E. tarda* and LPS challenge were investigated. To our knowledge, this is the IL-20L and IL-26 genes that have been reported for the first time in *Perciformes*. Our results confirmed that these four genes involve in the antimicrobial immune response of spotted sea bass.

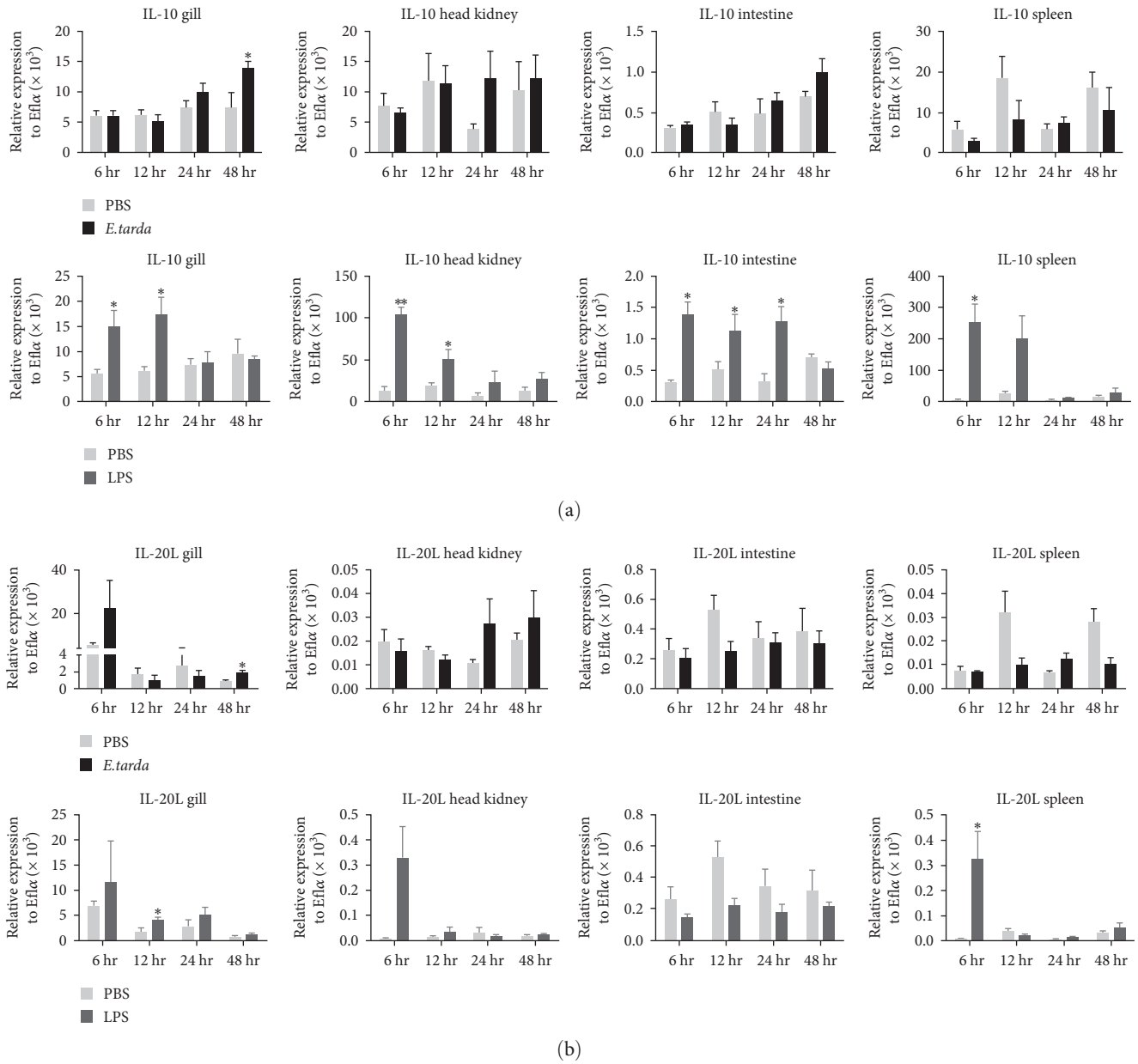


FIGURE 6: Continued.

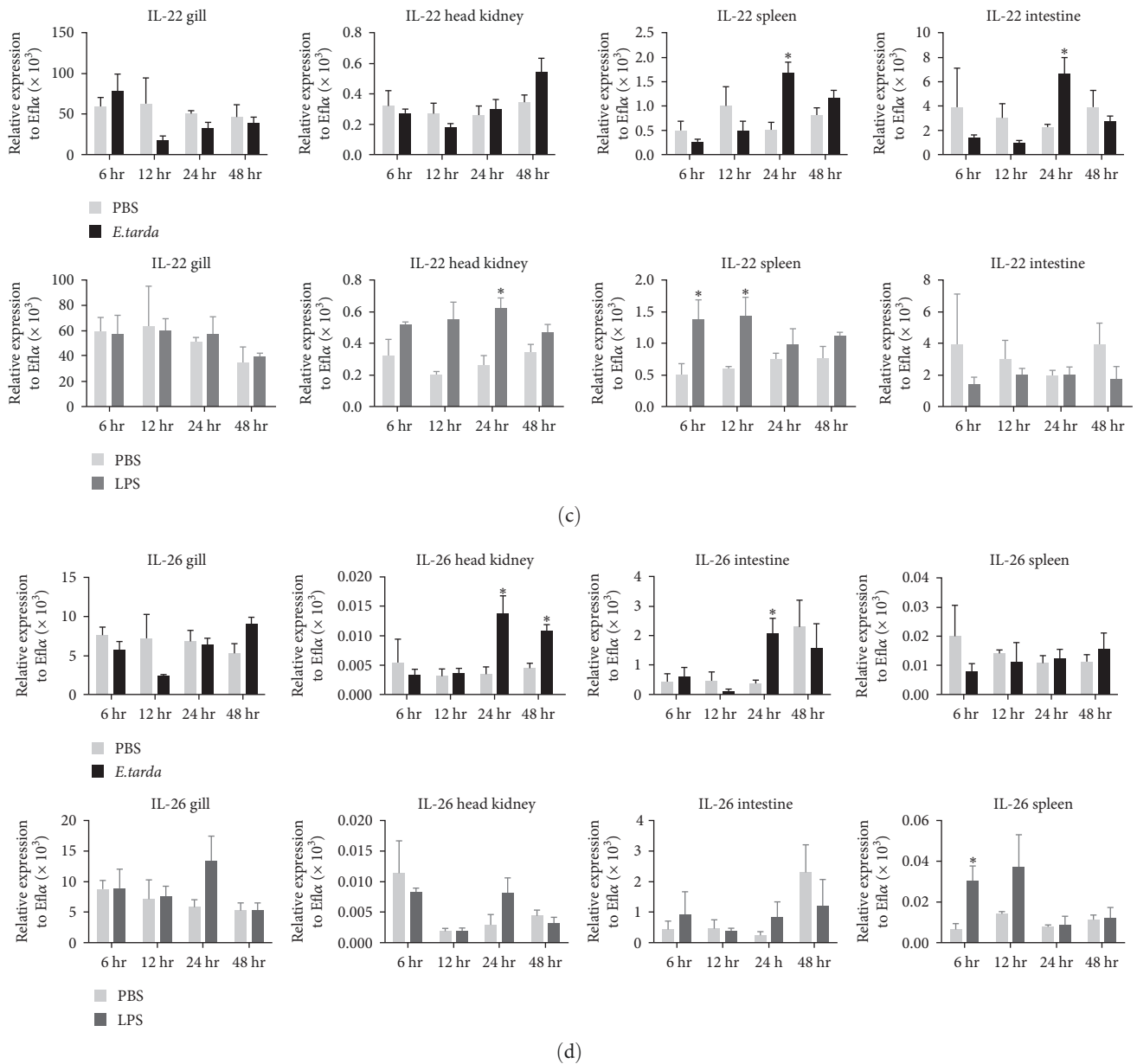


FIGURE 6: Analysis of (a) *LmIL-10*, (b) *LmIL-20L*, (c) *LmIL-22*, and (d) *LmIL-26* expression after infection of *E. tarda* and LPS. Spotted sea bass were injected with 300 μ L of *E. tarda* (1×10^4 CFU/mL in PBS) or equal volume of LPS and PBS (control). The gills, head kidney, intestine, and spleen were collected at 6, 12, 24, and 48 hr after injection. The *Efl α* gene was used as the internal reference gene. The results are shown as mean \pm SEM ($N = 4$), * for $0.01 < P < 0.05$, ** for $P < 0.01$.

Data Availability

Data are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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