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Research article

Cytochrome P450 superfamily in spotted sea bass: Genome-wide identification and expression profiles under trichlorfon and environmental stresses

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ABSTRACT

Cytochrome P450s (CYPs), as one of the most diverse enzyme superfamilies in nature, play critical functions in antioxidant reactions against endogenous and exogenous compounds. In this study, we performed genome-wide characterization of CYP superfamily members and analyzed their expression patterns under several abiotic stresses in spotted sea bass, which is known as an economically important fish species in the Chinese aquaculture industry. A total of 55 CYP genes were identified and divided into 17 families within 10 clans. The analysis of phylogeny, gene structure, and syntenic relationships provided evidence for the evolution of CYP genes and confirmed their annotation and orthology. The expression of CYP genes was examined in the liver during trichlorfon stress using quantitative real-time PCR. The results showed that 20 tested CYP genes displayed significant mRNA expression changes, indicating that they may play crucial roles in the metabolism of trichlorfon and can be potential biomarkers for trichlorfon pollution. Moreover, by screening transcriptomic databases, 10, 3 and 19 CYP genes exhibited differential expression patterns in response to hypoxia, alkalinity and heat stress, respectively. Taken together, this study provided insights into the regulation of CYP genes by toxicological and environmental stresses, laid basis for extensive functional studies of the CYP superfamily in spotted sea bass and other teleost species.

1. Introduction

Cytochrome P450s (CYPs) are a class of ligands binding to heavy metals named for their characteristic of spectral property with an absorption peak at 450 nm when combined with carbon monoxide, and they form a widely diverse superfamily of heme-thiolate enzymes (Werck-Reichhart and Feyereisen, 2000; Bernhardt, 2006; Hrycay and Bandiera, 2012). To date, >12,000 CYP genes have been discovered in living creatures ranging from bacteria, archaea, and viruses to higher plants and animals (Nelson, 2011; Guo et al., 2013). Based on sequence similarity and phylogenetic relationships, animal CYP genes are hierarchically grouped into three-level classifications, including clan, family and subfamily (Gotoh, 2012). Briefly, the "clan" is an upper category of CYP families that is useful for revealing the relationships among CYP genes in different phyla within each kingdom (Gotoh, 1993). CYP genes that share >40 % and 55 % amino acid sequence identity are considered to belong to the same family and subfamily, respectively (Nelson, 2006; Nelson, 2013; Nelson et al., 2013).

The CYP genes of vertebrates can be clustered into 19 families within 10 clans: clan 2 (CYP1, CYP2, CYP17 and CYP21 families), clan 3 (CYP3 and CYP5 families), clan 4 (CYP4 family), clan 7 (CYP7, CYP8 and CYP39 families), clan 19 (CYP19 family), clan 20 (CYP20 family), clan 26 (CYP16 and CYP26 families), clan 46 (CYP46 family), clan 51 (CYP51 family) and the mitochondrial clan (CYP11, CYP24 and CYP27 families) (Nelson et al., 2004; Nelson, 2009; Nelson, 2011; Nelson et al., 2013). The genes in the CYP1-3 families and some CYP4 families are more diverse than those in other CYP families, and they are mainly involved in the detoxification processes of exogenous substrates, such as pharmaceuticals (Guengerich, 2008; Nelson, 2013). The genes in the CYP5–51 families are primarily engaged in the synthesis or metabolism of endogenous molecules, such as eicosanoid and cholesterol (Denisov et al., 2005; Zhang et al., 2014; Lee et al., 2018). Overall, CYPs, as a class

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of enzymes with multiple physiological functions, their activities differ in terms of the regulation of gene expression, genetic polymorphism and environmental factors (Nair et al., 2016).

The study of CYPs in teleost species started relatively late, however, with rapid advances in sequencing technology, a growing number of fish CYP genes have been identified in several species, including Japanese pufferfish (*Fugu rubripes*) (Nelson, 2003), zebrafish (*Danio rerio*) (Dong et al., 2009; Goldstone et al., 2010), marine medaka (*Oryzias melastigma*) (Willett et al., 2006; Rhee et al., 2013) and channel catfish (*Ictalurus punctatus*) (Zhang et al., 2014). CYPs have been proven to play a key role in the detoxification and biotransformation of organophosphorus pesticides in aquatic animals (Uno et al., 2012; Kim et al., 2014). In addition, their potential involvement in the detoxification of various xenobiotic substances has been investigated by analyzing gene expression patterns. In the aquaculture industry, organophosphorus pesticides were often used to eliminate the threat of various fish parasites and minimize mortality and economic losses, with trichlorfon (2,2,2-trichloro-1-hydroxyethyl phosphonate) being most commonly used (Castro et al., 2021; Cruz et al., 2022; Silva et al., 2022). However, its toxicity to nontarget cultured fish species has been widely reported (Paulsen, 2020; Oliveira-Lima et al., 2021; Wang et al., 2022). Trichlorfon induces metabolic changes in aquatic animals, which can create widespread oxidative stresses and disturbances in enzymatic activities during several fundamental physiological processes (Ma et al., 2018). However, the molecular mechanisms underlying the detoxification of trichlorfon in fish remain largely unknown. Given the crucial roles of CYPs in the metabolism of xenobiotic compounds, it is necessary to explore the detoxification mechanisms of CYPs upon trichlorfon exposure in teleosts.

It is inescapable to suffer from external environment stressors in the process of fish growth and development. Previous study proved that CYP genes function in response to not only chemical compounds but also stress caused by various environmental factors. For instance, temperature is one of the most important abiotic factors, some thermal challenge experiments were performed on black rockfish (*Sebastes schlegelii*) (Lyu et al., 2018), rainbow trout (*Oncorhynchus mykiss*) (Rebl et al., 2013) and Atlantic salmon (*Salmo salar*) (Kang et al., 2018), several CYP genes were highlighted for their remarkable changes after heat stress. Moreover, there are also some functional characterizations of CYP genes response to hypoxia stress that have been reported in juvenile Amur sturgeon (*Acipenser schrenckii*) (Ni et al., 2014), cobia (*Rachycentron canadum*) (Yang et al., 2022), *Hypophthalmichthys molitrix* (Li et al., 2022) and silver sillago (*Sillago sihama*) (Saetan et al., 2020).

Spotted sea bass (*Lateolabrax maculatus*) is a commercially important marine-culture fish species in China because of its pleasant taste and high nutritive value, with annual production reaching 195.25 thousand tons in 2020 (Ministry of Agriculture and Rural Affairs, 2021). In this study, we performed genome-wide identification of CYP genes in spotted sea bass and examined their gene structures and evolutionary relationships. In addition, the expression change of CYP genes after trichlorfon treatment was detected to investigate their potential detoxification mechanisms. Furthermore, RNA-Seq data were utilized to screen the expression pattern of CYPs in response to several environmental stressors, including hypoxia, alkalinity and heat. Our findings may provide insights into the physiological roles of CYPs in spotted sea bass and lay a foundation for future in-depth evolutionary and functional studies of this superfamily in teleosts.

2. Materials and methods

2.1. Ethics statement

All research experiments involving in this study were approved by the Ethics Committee of Ocean University of China (Permit Number: 20141201). Fish were anesthetized by eugenol bath (200 mg/L) for several minutes to ensure that the welfare of tested fish will be well respected. Experiments involving fish are not endangered or conserved species.

2.2. Identification of CYP gene family in spotted sea bass

The CYP gene sequences of humans (*Homo sapiens*), zebrafish, channel catfish and fugu were retrieved from the Ensembl (http://www.ensembl.org), NCBI (http://www.ncbi.nlm.nih.gov/) databases and published articles (Nelson, 2003;Goldstone et al., 2010;Zhang et al., 2014), and were used as the query sequences to search against the transcriptome sequences (PRJNA347604) and the whole genome databases (PRJNA408177) of spotted sea bass with TBLASTN alignment tool (*E*-value = 1e-5). Subsequently, the candidate CYPs genes of spotted sea bass were aligned to whole-genome sequences with the BLASTN alignment tool (*E*-value = 1e-10) to verify the gene copy numbers after removing the redundant data. Open reading frame (ORF) were predicted and translated into amino acid sequences by ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/), and the results were validated by BLASTP against NCBI non-redundant protein (NR) database. The molecular weight (MW) and theoretical isoelectric points (pI) of the CYP genes were predicted by ExPASy-ProtParam (https://web. expasy.org/protparam/). Subcellular localization of CYP proteins was predicted using the Euk-mPLoc server (Chou and Shen, 2010).

2.3. Phylogenetic analysis

Phylogenetic analysis was carried out using the amino acid sequences of CYP genes of spotted sea bass and several representative animals retrieved from NCBI, Ensembl database and Cytochrome P450 Homepage (http://drnelson.uthsc.edu/CytochromeP450.html) (Nelson, 2009). Sequence alignment was performed by the ClustalW program, and the neighbor-joining method was implemented to construct the phylogenetic tree using MEGA 7 with the following parameters: JTT (Jones-Taylor-Thornton) and gamma distributed rate with invariant sites (G + I) model, bootstrapping with 1000 replications.

2.4. Structure analysis of spotted sea bass CYP genes

NCBI-CDD (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) was used to predict the conserved domain of CYP genes, and motifs were identified by the MEME suite (https://meme-suite.org/meme/index.html) (Marchler-Bauer et al., 2017) using the default parameters apart from the maximum number of motifs, which was set to 10. The images of CYP gene structures were visualized with TBtools (Chen et al., 2020).

2.5. Chromosomal distribution, gene duplication and syntenic analysis

The chromosomal locations of CYP genes of spotted sea bass were exhibited based on physical location information from the genome of spotted sea bass using Circos software (Krzywinski et al., 2009). Multiple Collinearity Scan toolkit (MCScanX) was applied to evaluate the gene duplication events with the default parameters (Wang et al., 2012). The collinear relationship of the orthologous CYP genes obtained from spotted sea bass and several teleosts, including zebrafish, large yellow croaker (*Larimichthys crocea*), Atlantic salmon, channel catfish and medaka, were investigated, and the syntenic maps were constructed using the Dual Systeny Plotter software (https://github.com/CJ-Chen/TBtools).

2.6. Trichlorfon treatment experiment

Before the formal experiment, the median lethal concentrations (LC_{50}) and safe concentrations (SC) of the trichlorfon were determined according to the results generated from a preliminary experiment. In detail, after a month of acclimation to the laboratory condition with

seawater temperature (14 ± 0.5 °C), pH (7 ± 0.5), and salinity (27–30 ‰), fish individuals (body length: 9.14 ± 0.19 cm, body weight: 14.99 ± 0.84 g) were starved for 24 h before exposure to trichlorfon. Trichlorfon (80.0 % soluble powder, Shanxi Zhengyue Chemical Pharmaceutical Co., Ltd., Shanxi, China) was formulated as a stock solution for the experiment. Twelve circular plastic tubs with 150 L capacity were used, and 20 fish were placed into each tub with 100 L of seawater, which contained different concentrations of trichlorfon (0, 0.200, 0.339, 0.574, 0.937 and 1.648 mg/L) with equal logarithmic spacing. Two replicated groups were settled for each concentration. The mortality/survival of fish in the tubs was recorded at four time-points (24 h, 48 h, 72 h and 96 h) after exposure to trichlorfon. The LC_{50} of the trichlorfon was determined using computational formulas: $LC_{50} = lg^{-1}[Xm - i(\Sigma P - 0.5)]$, Xm was the highest log concentration value, i was the logarithmic difference between two adjacent groups, and ΣP was the sum of mortality ratios (Karber, 1931). According to the Turubell formula (Hu et al., 2013), SC were calculated using the LC_{50} of 24 h and 48 h following the formulas: $SC = 48\text{ h } LC_{50} * 0.3 / (24\text{ h } LC_{50} / 48\text{ h } LC_{50})^2$.

For the formal experiment, after a month of acclimation to the water condition with temperature (14 ± 0.5 °C), pH (7 ± 0.5), and salinity (27–30), 250 fish individuals from the same pond (body length: 9.14 ± 0.19 cm, body weight: 14.37 ± 0.61 g) were used for the trichlorfon treatment experiment, SC (low toxicity, safe concentration) and LC_{50} (high toxicity, median lethal concentration of 48 h) groups were designed as treatment group at a density of 40 fishes per tank with 150 L of seawater, three replicates were established for both two treatment groups. Three individuals per tank were sampled at each time point, including 0 h (control group), 3 h, 6 h, 12 h, 24 h and 48 h after trichlorfon treatment. Liver tissues were dissected after anesthesia with a eugenol bath (200 mg/L), then flash-frozen in liquid nitrogen rapidly and stored at −80 °C for RNA extraction.

2.7. RNA extraction and quantitative real-time PCR (qPCR) analysis

The total RNA for each sample collected from the trichlorfon challenge experiment was extracted using TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions and digested with RNase-free DNase I (Takara, Otsu, Japan) to remove genomic DNA contaminations. RNA concentration and integrity were measured using Biodropsis BD-1000 spectrophotometric absorbance (Beijing Oriental Science & Technology Development Ltd., Beijing, China) and 1.5 % agarose gel electrophoresis (AGE). cDNA synthesis was performed using PrimeScript™ RT reagent Kit (Takara, Otsu, Japan) following the manufacturer's instructions. Expression levels of CYPs mRNA were detected by qPCR on the Applied Biosystems 7300 machines (Applied Biosystems, CA, USA). The primers used in this paper were designed by Primer 6 software and NCBI Primer-Blast tools. The qPCR amplicon size were set between 100 and 300 bp and the amplicon efficiency of the qPCR reaction reached 90 % were considered as appropriate primers, the detailed information of which were listed in Supplementary Table S1. 18S mRNA served as an internal reference gene. The $2^{-\Delta\Delta CT}$ method was utilized to calculate the relative gene expression values, and one-way ANOVA and Waller-Duncan multiple testing were performed using SPSS software. The differences were considered statistically significant at P values <0.05.

2.8. Expression patterns of CYPs in response to environmental stressors by the screening of RNA-Seq data sets

RNA-Seq reads were retrieved from three transcriptome sequencing databases to explore the mRNA expression changes of the CYP gene family in response to alkalinity, hypoxia and heat stress conditions. (1) For the alkalinity treatment experiment, one-year-old spotted sea bass (body weight: 140.32 ± 2.56 g) were acclimated for four weeks in freshwater (pH: 7.8 ± 0.4), and the carbonate-alkalinity solution was previously made up of $NaHCO_3$ (12.8 mmol/L) and Na_2CO_3 (2.6 mmol/L) in fresh water and aerated for 24 h. Then 45 spotted sea bass individuals were randomly divided into three replicates for the alkalinity treatment experiment at a density of 15 fish per tank with 100 L of carbonate-alkalinity solution. During the period of the stress experiment, no feeding was performed, and carbonate-alkalinity solution was measured daily with acidimetric titration to maintain alkalinity at 18 ± 0.2 mmol/L, meanwhile the dissolved oxygen concentration, temperature and pH were kept at 7.1 ± 0.4 mg/L, 22 ± 1 °C and 9.0 ± 0.2, respectively. The gill tissues of three individuals from each replicated tank were sampled at 0 h (before alkalinity exposure) and 12 h, 24 h and 72 h after alkalinity stress, and then rapidly frozen in liquid nitrogen. RNA of three individuals from the same replicate was mixed into one sample, and then 12 sequencing libraries were established. 150 bp paired-end sequencing data were generated by Illumina HiSeq X Ten platform (PRJNA611641). (2) For the hypoxic challenge experiment, a total of 60 fish individuals (body weight: 178.25 ± 18.56 g, body length: 48.76 ± 4.26 cm) were acclimated for two weeks at water condition with temperature (17.0 ± 1.0 °C), dissolved oxygen (7.0 ± 0.5 mg/L), pH (6.5–7.5). After that, 9 individuals were randomly collected and considered as control group (0 h), and the remaining 51 individuals were randomly divided into three replicates for the hypoxic treatment experiment at a density of 17 fishes per tank. Dissolved Oxygen (DO) concentration in the hypoxic group was decreased to 1.1 ± 0.14 mg O_2/L by aerating with nitrogen gas and maintaining the DO concentration constant during the treatment. The gill tissues of three individuals per replicated tank were sampled at 0 h (control group), 3 h, 6 h, and 12 h after exposure. After quick freezing in liquid nitrogen, these samples were stored at −80 °C for RNA extraction. Equal quantity of RNA from the same time-point was pooled to one sample, and then we conducted 12 sequencing libraries, 150 bp paired-end sequencing data were generated by Illumina HiSeq PE150 platform. (3) In the heat stress experiment, 60 spotted sea bass individuals (body weight: 38.96 ± 2.01 g, body length: 13.33 ± 0.24 cm) were randomly collected and acclimated for two weeks at experiment condition with temperature (25.0 ± 1.0 °C), dissolved oxygen (7.0 ± 0.5 mg/L), pH (7.5 ± 0.4) and salinity (31 ± 1.0 ‰). After acclimation, 60 individuals were divided into three replicates at the density of 20 per tank, and the temperature of seawater was increased from 25 to 32 °C at a rate of 1 °C per hour. The temperature (32 °C) was maintained until the end of the heat stress experiment, meanwhile the liver tissues of three individuals per tank were sampled at 0 h, 3 h, 6 h, 12 h and 24 h during heat stress. Tissues were frozen in liquid nitrogen and stored at −80 °C for RNA extraction. RNA of three individuals from the same replicate was mixed into one sample, and then 15 sequencing libraries were constructed. 150 bp paired-end sequencing data were generated by Illumina Novaseq 6000 platform.

All of the above three transcriptomic data sets were used for differential expression analysis of CYP genes after environmental stresses. For each data set, high-quality clean reads were acquired by removing adapters and low-quality reads using Trimmomatic v0.36 software (Bolger et al., 2014), and then clean reads were aligned to the genome of spotted sea bass (PRJNA408177) via HISAT2 v2.2.1 (Kim et al., 2015) alignment program with default parameters. The number of reads matched to protein-encoded genes in each sample was counted by using the FeatureCounts program from the Subread package v2.03 (Liao et al., 2014). Gene expression profiling was normalized by the value of fragments per kb of exon model per million mapped reads (FPKM) by StringTie v2.1.4 software (Pertea et al., 2016). DESeq 2 v1.36 (R package) was executed to identify differentially expressed genes (DEGs) with a threshold of $\log2|(\text{fold change})| \geq 1$, adjusted $P \leq 0.05$, and total reads ≥5.

3. Results

3.1. Identification and characterization of CYP genes in spotted sea bass

A total of 55 CYP genes were identified in the spotted sea bass and

named based on the rules for CYP gene nomenclature (Table 1) (Nelson, 2006). These CYP genes were classified into 10 clans: clan 2, clan 3, clan 4, clan 7, clan 19, clan 20, clan 26, clan 46, clan 51 and the mitochondrial clan. The 10 clans were further classified into 17 families, including CYP1, CYP2, CYP3, CYP4, CYP5, CYP7, CYP8, CYP11, CYP17, CYP19, CYP20, CYP21, CYP24, CYP26, CYP27 CYP46 and CYP51. Among the 17 CYP families, CYP1 (5 genes), CYP2 (17 genes) and CYP27 (6 genes) were the three dominant families (28 CYP genes among them), accounting for more than half of the total CYP genes in spotted sea bass. However, unique gene members were identified in the CYP5, CYP20, CYP21, CYP24, CYP46 and CYP51 families. In detail, the predicted molecular weight of spotted sea bass CYP proteins ranged from 24.05 kDa to 89.64 kDa, and the deduced isoelectric point ranged from 5.20 to 9.71. The CYP21A1 gene had the smallest molecular weight of 24.05 kDa, and the CYP17A1 gene possessed the largest molecular weight of 89.64 kDa. The open reading frames (ORFs) of CYP genes ranged from 657 to 2406 bp, with predicted protein lengths ranging from 218 to 801 amino acids (aa). Subcellular location prediction uncovered that 39 CYP proteins were uniquely anchored in the endoplasmic reticulum (ER), and two CYP proteins were positioned in the mitochondrion. Two possible subcellular localizations were simultaneously predicted for 14 CYP proteins. One was the endoplasmic reticulum, and the other was the microsome, cytoplasm or nucleus. The cDNA sequences of these CYP genes have been submitted to the GenBank database, and the characteristics of these genes are summarized in Table 1.

Table 1
Basic information of CYP genes in spotted sea bass.

Clan name	Family name	Gene name	ORF length (bp)	Predicted protein length (aa)	MW (kDa)	PI (isoelectric point)	Predicted subcellular localization	Accession number of NCBI
clan 2	CYP1	*CYP1A1*	1563	520	58.92	6.29	ER[a], microsome	MN685617
		CYP1B1	1605	534	60.75	8.80	ER	MN685618
		CYP1C1	1578	525	59.08	8.26	ER	MN685619
		CYP1C2	1575	524	68.69	6.99	ER	MN685620
		CYP1D1	1587	528	59.76	7.61	ER	MN685621
	CYP2	*CYP2D15*	1329	442	50.83	7.19	ER	MN685622
		CYP2F2A	1077	358	36.48	6.22	ER	MN685623
		CYP2F2B	960	319	41.08	5.20	ER	MN685624
		CYP2F2C	1230	409	47.19	5.50	ER	MN685625
		CYP2F2D	1230	409	47.11	5.50	ER	MN685626
		CYP2F3	1473	490	55.93	8.59	ER	MN685627
		CYP2G1	1476	491	56.61	8.89	ER	MN685628
		CYP2J2B	1482	493	56.60	5.81	ER	MN685629
		CYP2J2A	1503	500	57.40	7.23	ER	MN685630
		CYP2J6A	969	322	37.05	6.60	ER	MN685631
		CYP2J6B	753	250	28.42	5.83	ER, cytoplasm	MN685632
		CYP2K1A	2349	782	89.28	7.89	ER, microsome	MN685633
		CYP2K1B	1380	459	51.79	7.10	ER	MN685634
		CYP2K1C	708	235	26.90	9.71	ER	MN685635
		CYP2N14	1494	497	56.32	6.55	ER	MN685636
		CYP2R1	1560	519	58.70	8.85	ER, microsome	MN685637
		CYP2U1	1590	529	59.06	6.81	ER	MN685638
	CYP17	*CYP17A1*	2406	801	89.64	9.05	ER, nucleus	MN685654
		CYP17A2	1281	426	46.97	9.31	ER	MN685655
	CYP21	*CYP21A1*	657	218	24.05	6.05	ER	MN685659
clan 3	CYP3	*CYP3A*	1512	503	57.96	7.01	ER, microsome	MN685639
		CYP3A30	1530	509	58.04	6.44	ER, microsome	MN685640
		CYP3A56	1497	498	57.40	7.60	ER	MN685641
		CYP3A40	1494	497	57,23	7.98	ER	MN685642
	CYP5	*CYP5A1*	1689	562	64.69	7.93	ER	MN685645
clan 4	CYP4	*CYP4F3*	1599	532	60.99	7,10	ER	MN685643
		CYP4V2	1239	412	47.41	6.28	ER	MN685644
clan 7	CYP7	*CYP7A1*	1542	513	58.84	7.05	ER	MN685646
		CYP7B1	1500	499	57.52	8.69	ER	MN685647
	CYP8	*CYP8A1A*	1443	480	55.08	8.94	ER	MN685648
		CYP8A1B	1470	489	56.47	8.79	ER	MN685649
		CYP8B1A	1536	511	59.23	8.91	ER	MN685650
		CYP8B1B	729	242	28.13	8.84	ER	MN685651
clan 19	CYP19	*CYP19A1*	1557	518	58.72	7.61	ER	MN685656
		CYP19A2	1506	501	56.93	8.30	ER	MN685657
clan 20	CYP20	*CYP20A1*	1395	464	51.74	6.11	ER	MN685658
clan 26	CYP26	*CYP26A1*	1539	512	35.20	6.05	ER, microsome	MN685661
		CYP26B1	927	308	57.71	8.65	ER, microsome	MN685662
		CYP26C1	1623	540	60.49	8.08	ER	MN685663
clan 46	CYP46	*CYP46A1*	1227	408	46.60	6.68	ER	MN685670
clan 51	CYP51	*CYP51A1*	1500	499	56.52	7.21	ER	MN685671
mitochond-rial clan	CYP11	*CYP11A1*	1557	518	59.86	9.17	Mitochondrion	MN685652
		CYP11B	1503	500	55.78	9.51	ER	MN685653
	CYP24	*CYP24A1*	1542	513	58.41	9.14	Mitochondrion	MN685660
	CYP27	*CYP27A1*	1539	512	58.97	6.81	ER, mitochondrion	MN685664
		CYP27A3	1821	606	68.59	9.38	ER, mitochondrion	MN685665
		CYP27A2	1602	533	60.37	8.49	ER, mitochondrion	MN685666
		CYP27B1	1530	509	57.68	9.20	ER, mitochondrion	MN685667
		CYP27C1A	1626	541	61.06	6.90	ER	MN685668
		CYP27C1B	1626	541	61.20	6.72	ER, mitochondrion	MN685669

[a] ER: endoplasmic reticulum.

3.2. Phylogenetic and gene copy number analysis of CYPs

To investigate the evolutionary relationship of CYP genes, a phylogenetic tree was constructed based on the predicted CYP amino acid sequences from spotted sea bass, representative mammals (human, mouse, dog and cow) and teleosts (zebrafish, fugu, and channel catfish). As shown in Fig. 1, the CYP genes of spotted sea bass were grouped into 10 separate clans, including clan 2, clan 3, clan 4, clan 7, clan 19, clan 20, clan 26, clan 46, clan 51 and the mitochondrial clan, which is consistent with the classification described in previous research. The results showed that all members of CYP genes were well clustered with the expected evolutionary clan and were supported by robust bootstrap values. Clan 2 was the largest, consisting of 25 genes from four gene families (CYP1, CYP2, CYP17 and CYP21). Clan 20, clan 46 and clan 51 contained unique gene families.

The copy numbers of CYP genes in spotted sea bass and several representative vertebrates (mammals and teleosts) are compared and summarized in Supplementary Table S2. A total of 100 gene copies were detected in mice, which was the largest number among the eight representative species. Zebrafish ranked second, harboring 92 CYP gene copies. The copy numbers of the CYP gene ranged from 50 to 60 for the remaining six tested species. Among these CYP families, CYP2 has the largest subfamily and showed diversity in the number of members across the selected species. The CYP5, CYP17, CYP19, CYP20, CYP21, CYP24, CYP39, CYP46 and CYP51 families have only one subfamily in all selected species. Compared with teleosts, we discovered that the CYP2B,

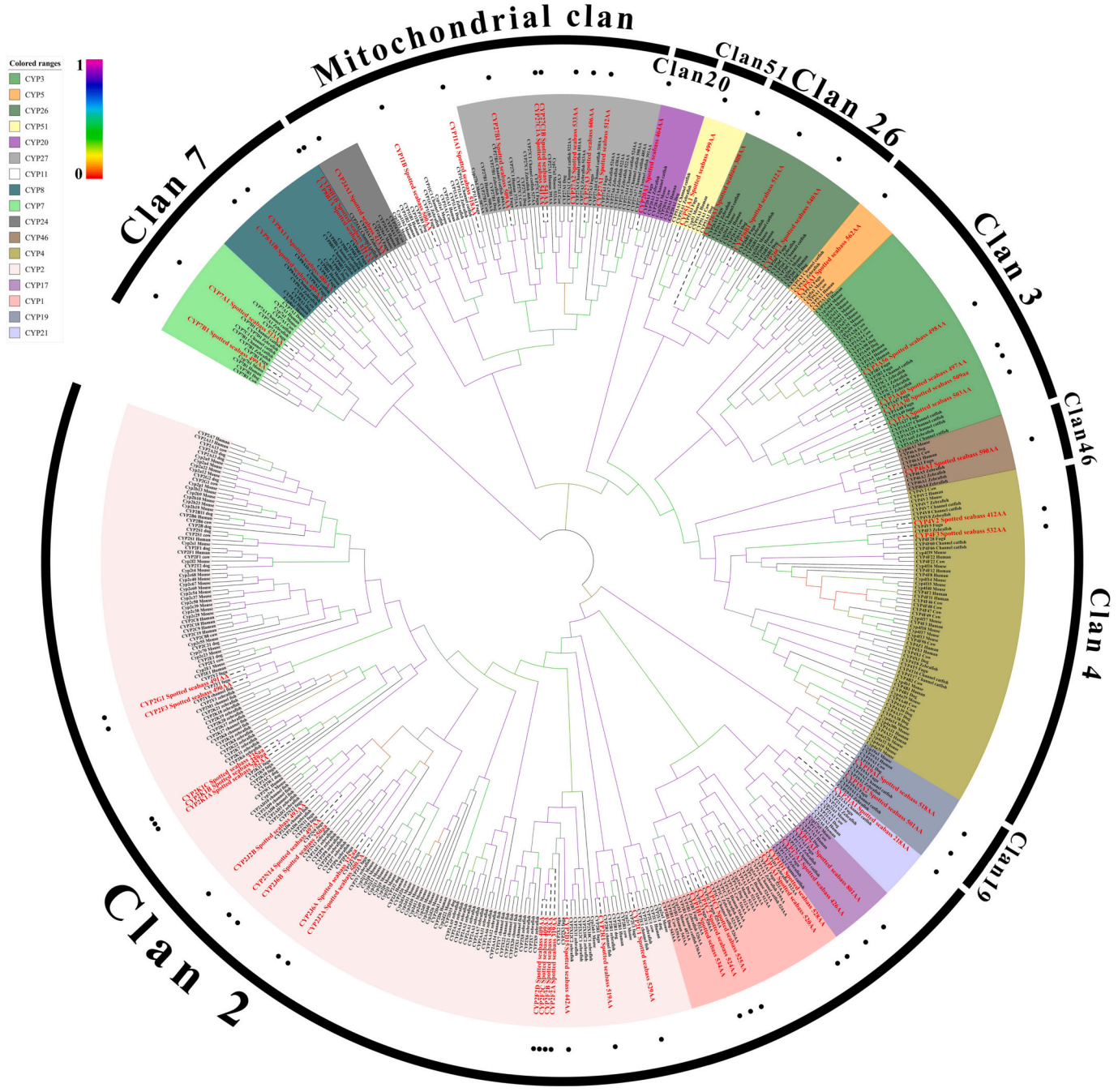

Fig. 1. Phylogenetic analysis of spotted sea bass CYP genes. The phylogenetic tree was constructed using amino acid sequences from several representative mammals and teleosts with 1000 bootstrap replications in MEGA 7. The CYP genes of spotted sea bass are labeled with black dots and red bold fonts. Different clade colors represent bootstraps. The phylogenetic tree was divided into 10 clans with covered lines and 17 families with different backgrounds.

CYP2C, CYP2E, CYP2S, CYP2T, CYP2W, CYP4A, CYP4B, CYP4X and CYP4Z subfamilies existed only in mammals, while the CYP1C, CYP1D, CYP2K, CYP2M, CYP2N, CYP2P, CYP2V, CYP2X, CYP2Y, CYP2Z, CYP3B, CYP3C, CYP4T, CYP7C, CYP7D and CYP11C subfamilies were specific to teleosts (Supplementary Table S2). Besides, the CYP4V, CYP8A, CYP8B, CYP11A, CYP17A, CYP19A, CYP27A, CYP27C and CYP46A subfamilies were expanded, and the CYP1A, CYP4F and CYP11B subfamilies were contracted in several teleost species compared with mammals. For example, for the CYP17A and CYP19A subfamilies, two copies were present in all tested teleosts, but mammals harbored only one copy. For spotted sea bass, the CYP2X, CYP4T and CYP7C subfamilies were lost in its genome, and the CYP2F, CYP2J and CYP27C subfamilies showed a significant expansion in copy number. Notably, spotted sea bass possessed CYP2D and CYP2G, which are subfamilies that have been annotated in several mammals but without homologous genes in teleosts (Supplementary Table S2).

3.3. Domains and motif analysis of spotted sea bass CYP genes

Based on the results of MEME suite analysis, 10 motifs were acquired among CYP genes of spotted sea bass (Fig. 2). Overall, CYP genes within the same clan shared similar motif structures. The number of the motifs in clan 2 was the largest, with a total of 2–9 motifs. Among them, motif 8 was the most conserved and shared among all clan 2 members. Clan 46 had the fewest motifs, comprising motif 1, motif 2, motif 3 and motif 4 only. All of the clans except clan 4 contained motif 1. In addition, motif 2, motif 3, motif 4, motif 5 and motif 7 were found in most clans. In addition, several specific motifs were observed within some clans. For instance, motif 9 was unique for clan 2 and the mitochondrial clan, and motif 10 existed in clan 2 alone. For clan 3, the only difference between CYP 3 and CYP 5 was that the CYP 5 members possessed motif 8, which was not identified in CYP 3 (Fig. 2).

Conserved domains were also predicted by NCBI-CDD. The P450 superfamily structural domain of CYP genes covered almost the full length of amino acids, indicating that the core region of the family was conserved. Outside the conserved structural domain, an additional motif 2 was predicted in the CYP26C1 gene. Notably, a novel COG5048 structural domain was detected in the *CYP17A1* gene, the function of which needs to be further investigated (Fig. 2).

3.4. Chromosomal distribution of CYP genes

The CYP genes of spotted sea bass were distributed on 20 chromosomes and 2 unanchored scaffolds (Fig. 3). Six CYP genes were identified on chr2 and chr6, respectively, which had the most CYP genes. In contrast, chr8, chr16, chr20, chr21, and chr23 possessed only one CYP gene. Generally, CYP genes from the same family were decentralized in the genome of spotted sea bass. For example, CYP1 family members were distributed on four chromosomes (chr1, chr8, chr13 and chr19). Gene density maps showed that 27 CYP genes were in the sense strand, and the remainder was in the antisense strand (Fig. 3).

According to the descriptions of Holub (Holub, 2001), a chromosomal region within 200 kb containing two or more genes is defined as a tandem duplication event. By MCScanX, 15 CYP genes (*CYP2J6B/2N14/2J2B/2J6A*, *CYP3A/3A40*, *CYP2F3/2G1*, *CYP2D15/2F2C/2F2D*, *CYP2K1B/2K1C*, and *CYP1C1/1C2*) were clustered into 6 tandem duplication event regions on spotted sea bass chr2, chr3, chr5, chr6, chr15, and chr19. These results indicated that tandem duplication contributes to the amplification of spotted sea bass CYP genes.

3.5. Synteny analysis

Syntenic analysis was performed to further explore the evolutionary relationship of the CYP genes in spotted sea bass and five representative teleosts, respectively (Fig. 4). The CYP genes in spotted sea bass were homologous to genes in the representative teleosts, and a higher level of syntenic conservation was observed among Atlantic salmon (42 pairs of orthologous gene pairs distributed on all chromosomes except chr7, chr9, chr12, chr14, chr17, chr18, chr20, chr21, chr22, and chr24), large yellow croaker (34 pairs of orthologous gene pairs distributed on all chromosomes except chr12, chr14, chr17, chr19, chr21, and chr22), medaka (*O. latipes*) (36 pairs of orthologous gene pairs distributed on all chromosomes except chr9, chr12, chr14, chr17, chr19, chr20, chr21, and chr22), channel catfish (17 pairs of orthologous gene pairs

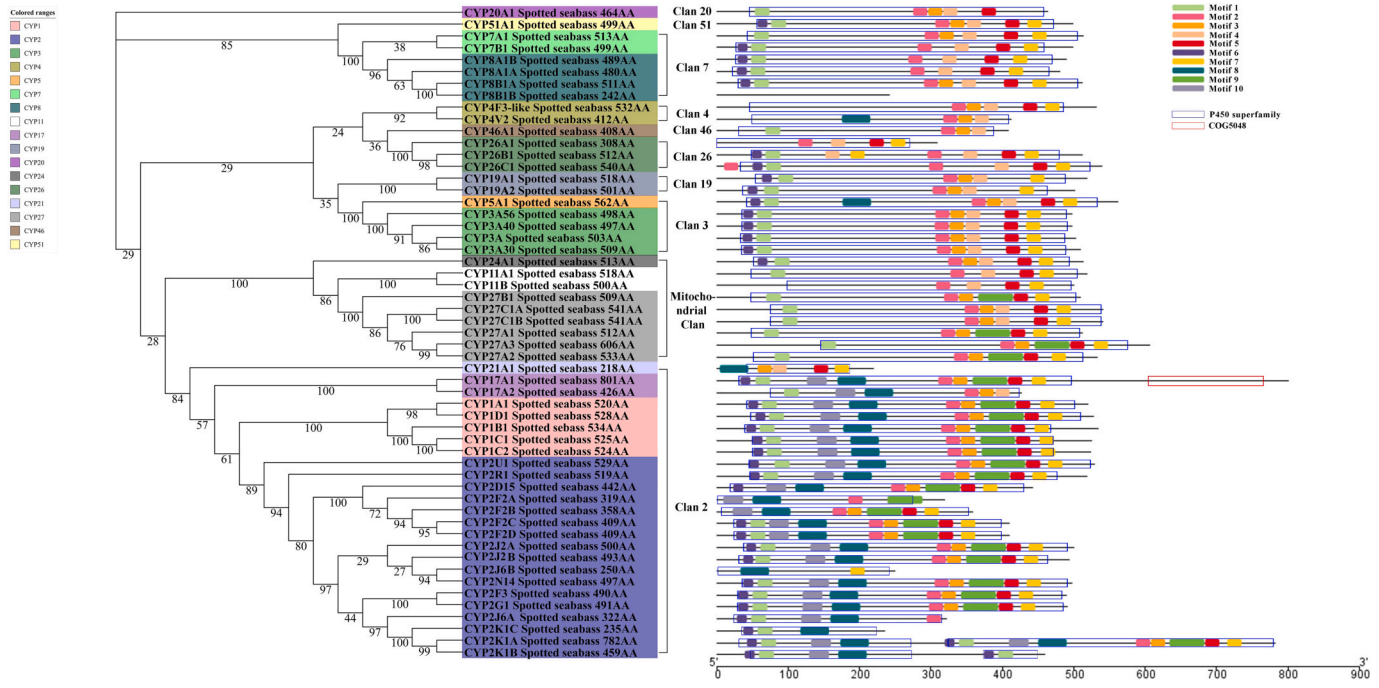

Fig. 2. Phylogenetic relationship and architecture of conserved protein motifs in CYP genes from spotted sea bass. CYP families are shown in different background colors, and genes belonging to the same clan are labeled. Motifs are displayed in different colored boxes. Domains are labeled in wire frames.

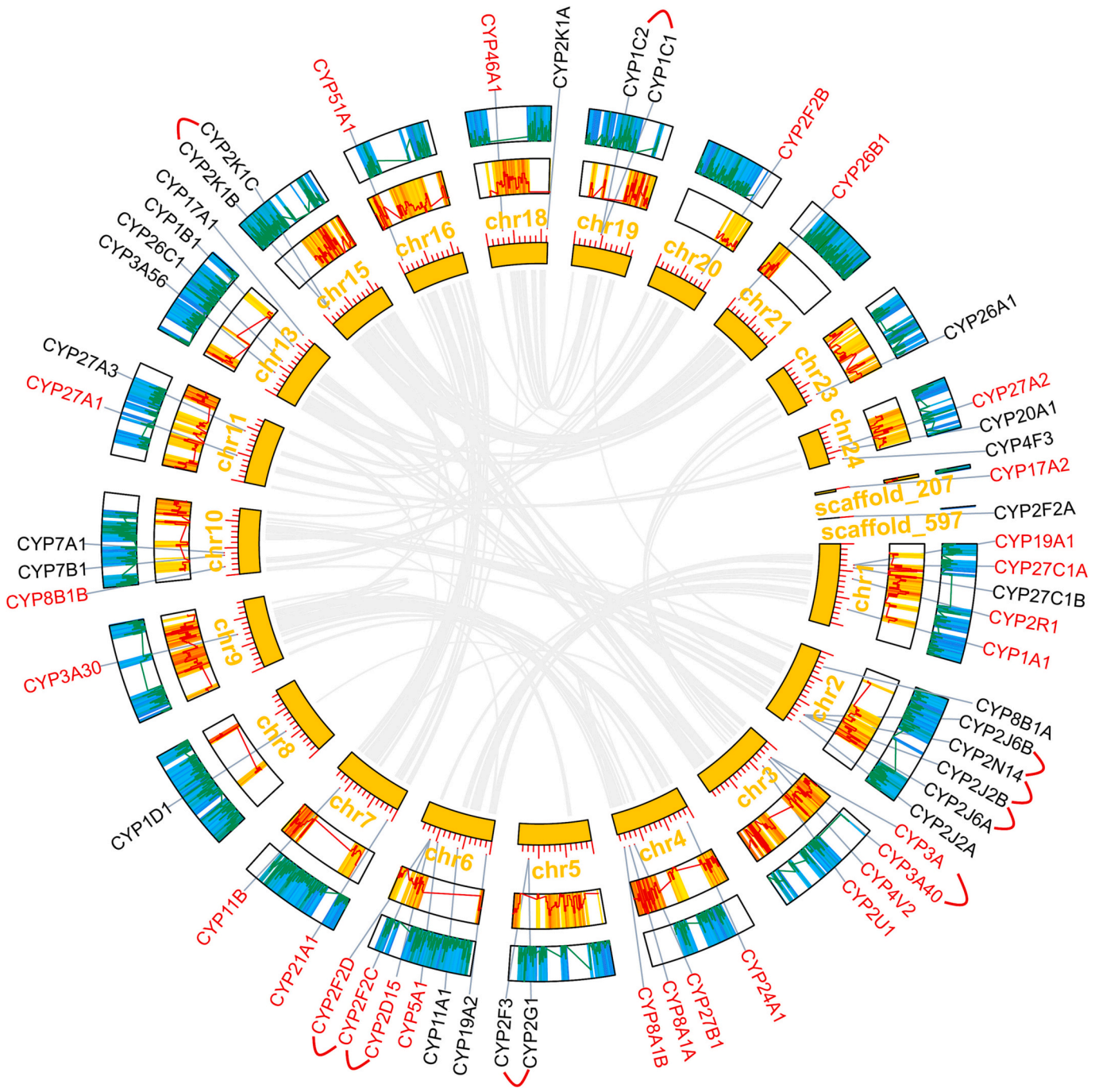

Fig. 3. Schematic representations of the chromosomal (scaffold) distributions and interchromosomal relationships of CYP genes. The gray line represents the whole genome collinear block of spotted sea bass mined by MCScanX, the red line represents the tandem repeat between CYP genes, the yellow square indicates the chromosome and the scaffold, and the outer ring indicates the gene density. CYP genes in sense strands are labeled in red, and antisense strands are labeled in black. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

distributed on chr1, chr2, chr3, chr4, chr5, chr6, chr11, chr13, and chr15) and zebrafish (16 pairs of orthologous gene pairs distributed on chr1, chr3, chr4, chr6, chr8, chr10, chr13, chr15, chr16, and chr19). In general, these results revealed that these CYP genes were relatively highly conserved among teleosts and that spotted sea bass was closer to Atlantic salmon than to zebrafish from the perspective of the evolutionary origin of CYP genes.

3.6. *Expression patterns of selected CYP genes after trichlorfon treatment*

According to the statistical analysis, the dose–response assay showed no mortality in the control group (0 mg/L) after 96 h of exposure. The mortality of experimental spotted sea bass under different concentrations of trichlorfon is listed in Supplementary Table S3. The resulting 24 h, 48 h, 72 h and 96 h median lethal concentration (LC_{50}) values for trichlorfon were 0.873, 0.786, 0.745 and 0.745 mg/L, respectively, which were responsible for 50 % mortality of the experimental fish. The safe concentration (SC) was calculated as 0.191 mg/L. Therefore, in this study, we conducted trichlorfon exposure experiments using the SC and LC_{50} (48 h) to explore the significant role of CYP in response to chemical substance stress.

Based on previous research, 20 CYPs belonging to the CYP1 to CYP4

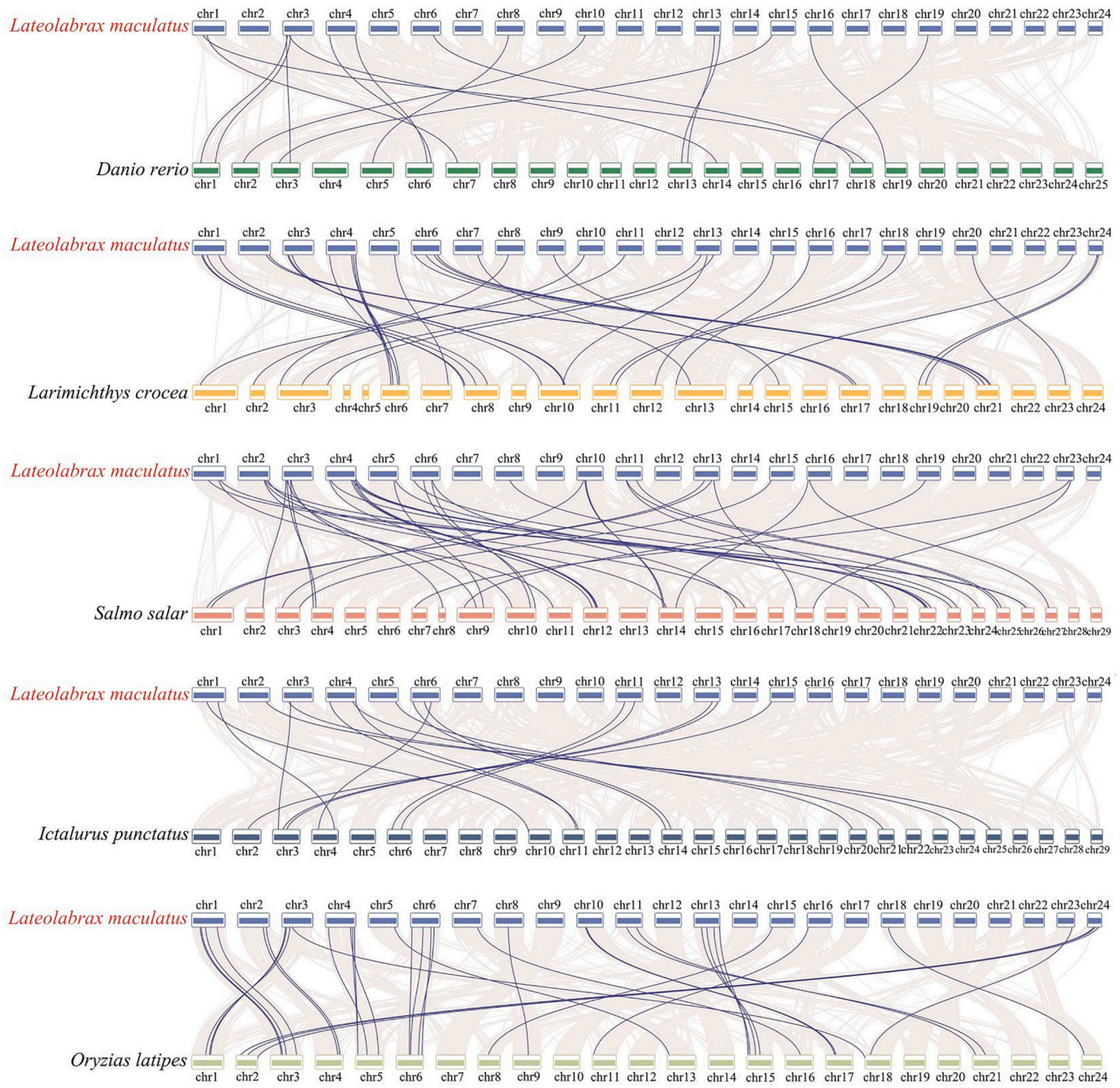

Fig. 4. Synteny analysis of CYP genes between spotted sea bass and five representative teleosts. Gray lines in the background represent the collinear blocks within spotted sea bass and other teleosts, while the blue lines highlight the syntenic CYP gene pairs. The teleosts used in synteny analysis included zebrafish, yellow croaker, Atlantic salmon, channel catfish and medaka. Different boxes represent chromosomes of corresponding species. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

families and reported to be highly involved in the metabolism of xenobiotics and drugs (Goldstone et al., 2010) were selected to detect the expression pattern after trichlorfon treatment in liver tissue. Overall, all detected CYP genes showed various changes in expression among the 6 time points in the liver of spotted sea bass (Fig. 5). In the SC group, the *CYP1A1*, *CYP1B1*, *CYP1C1*, *CYP1C2*, *CYP2K1A*, *CYP2U1* and *CYP4F3* genes showed a similar expression tendency of increasing at the beginning and then declining with time, demonstrating that trichlorfon treatment has an intensive effect on CYP gene expression. The maximum relative expression of the *CYP2D15*, *CYP2F2C*, *CYP2F3*, *CYP2N14*, *CYP3A*, *CYP3A30*, *CYP3A40*, *CYP3A56* and *CYP4V2* genes was observed at 48 h. For the *CYP2J2A* and *CYP2R1* genes, the relative expression was downregulated significantly at 3 h and 6 h but significantly up-regulated at 12 h, 24 h and 48 h. The expression patterns of *CYP2G1* and *CYP2J6A* genes were similar, with maximum relative expression at 12 h and showing a tendency of relatively down-regulated expression at the other time points. In the LC_{50} (48 h) group, the relative expression of the *CYP1C1*, *CYP2D15*, *CYP2F2C*, *CYP2F3*, *CYP2J2A*, *CYP2N14*, *CYP3A30* and *CYP3A56* genes was unvaried or even significantly down-regulated significantly at the time point before 24 h (including 24 h) compared with at 0 h but was significantly up-regulated at 48 h. The *CYP2G1*, *CYP2J6A*, *CYP2K1A*, *CYP2R1*, *CYP2U1*, *CYP4F3* and *CYP4V2* genes exhibited down-regulated expression tendencies at most time points. In contrast, the *CYP3A* and *CYP3A40* genes showed a global tendency of

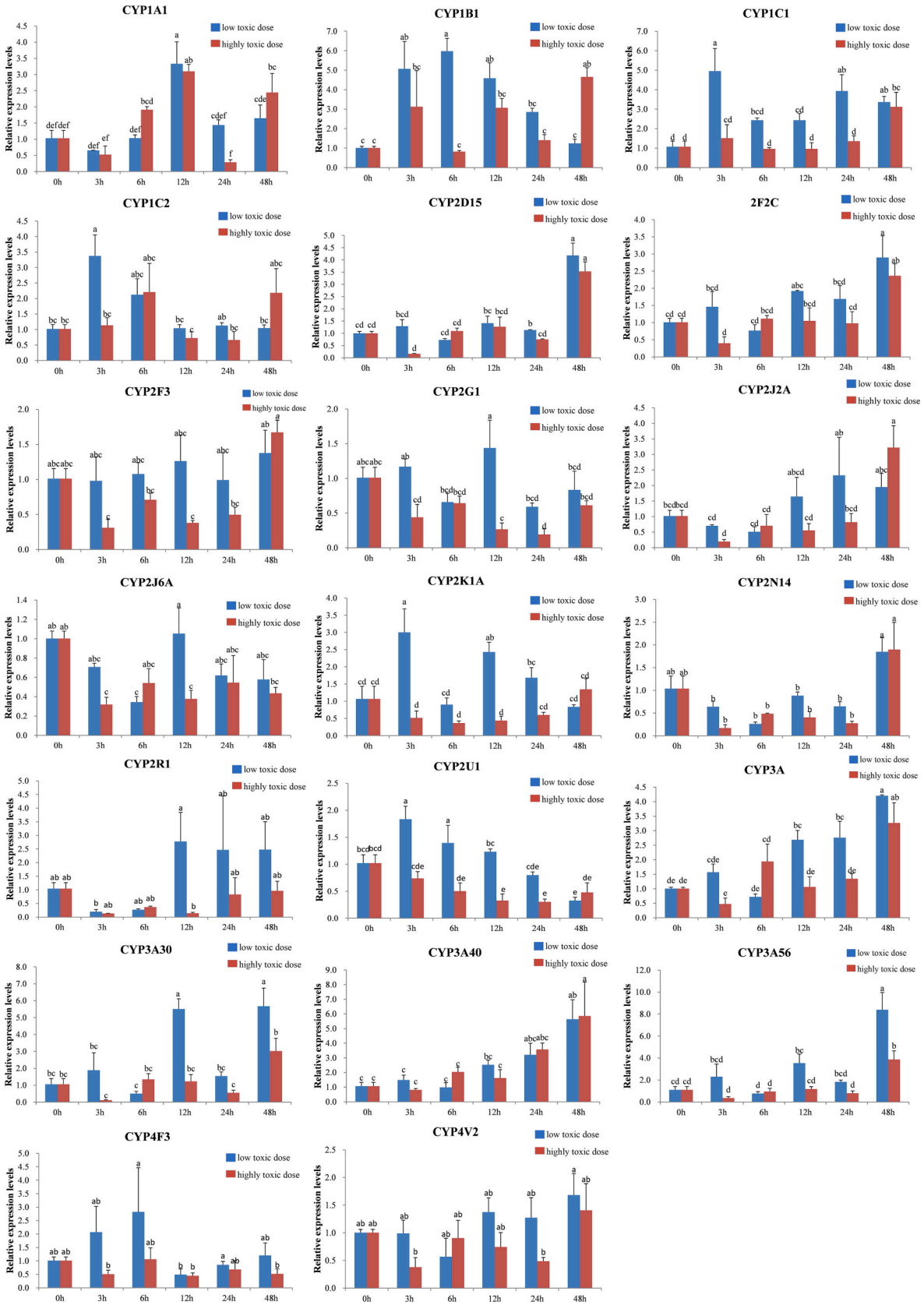

Fig. 5. Expression patterns of 20 CYP genes in the liver tissue of spotted sea bass after trichlorfon treatment. qRT–PCR analysis was used to determine the expression patterns of CYP genes at 0 h, 3 h, 6 h, 12 h, 24 h and 48 h after trichlorfon treatment. The relative expression was calculated by the control group (0 h) and normalized by 18S rRNA. The blue bar represents the low toxicity group (SC group, 0.191 mg/L), while the red bar represents the high toxicity group (LC_{50} of 48 h, 0.786 mg/L). Various letters on the column chart indicate a significant difference ($P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

up-regulated expression. On the whole, the relative expression of genes in the SC group was commonly higher than that in the LC50 (48 h) group at most time points, suggesting that spotted sea bass is more insensitive to low concentrations of trichlorfon.

3.7. Expression patterns of CYP genes in response to hypoxia, alkalinity and heat challenges

A transcriptomic analysis was conducted to investigate the expression profiles of CYP genes in response to environmental stressors. After the hypoxia stress, a total of 6 genes including the *CYP1A1*, *CYP2F2B*, *CYP2F3*, *CYP2G1*, *CYP7A1* and *CYP27C1B* genes were significantly up-regulated for at least one time point in the gill tissue. Among these genes, the expression of the *CYP2F2B* and *CYP2G1* genes was significantly up-regulated at all three detected time points (3 h, 6 h and 12 h); the *CYP1A1* and *CYP7A1* genes were significantly up-regulated at 6 h; and the *CYP2F3* and *CYP27C1B* genes were significantly up-regulated at 12 h. In contrast, 4 genes (*CYP1C1*, *CYP2K1A* , *CYP3A56* and *CYP4F3*) in the gill tissue of spotted sea bass were down-regulated significantly after the hypoxia challenge for at least one time point. Among these genes, the expression of the *CYP4F3* gene was significantly down-regulated at all three detected time points (3 h, 6 h and 12 h), and the expression pattern of the *CYP1C1* gene was significantly down-regulated at 3 h and 12 h. The remaining 2 genes (*CYP2K1A* and *CYP3A56*) were significantly down-regulated expression at 6 h and 12 h respectively.

After alkalinity stress, the *CYP2G1* gene appeared to be significantly up-regulated only at 72 h in gill tissue. No gene was significantly up-regulated at 12 h and 24 h. In addition, 2 CYP genes (*CYP8A1A* and *CYP51A1*) were remarkably down-regulated for at least one time point after stress, especially the *CYP51A1* gene was significantly down-regulated at all tested time-points, and *CYP8A1A* gene was significantly down-regulated only at 12 h and 24 h. In the liver tissue treated with heat stress, 19 genes showed significant changes after heat stress, of which 12 genes (*CYP1C1*, *CYP1C2*, *CYP2F2A*, *CYP2F2C*, *CYP2K1B*, *CYP2R1*, *CYP2U1*, *CYP3A*, *CYP3A40*, *CYP5A1*, *CYB7B1* and *CYP26A1*) were significantly up-regulated and 7 genes (*CYP2F3*, *CYP2G1*, *CYP2J2A*, *CYP2J2B*, *CYP27A3*, *CYP27C1B* and *CYP51A1*) were significantly down-regulated. The number of up-regulated expressed genes was greater than that of down-regulated genes.

Overall, a total of 26 CYP genes of spotted sea bass showed

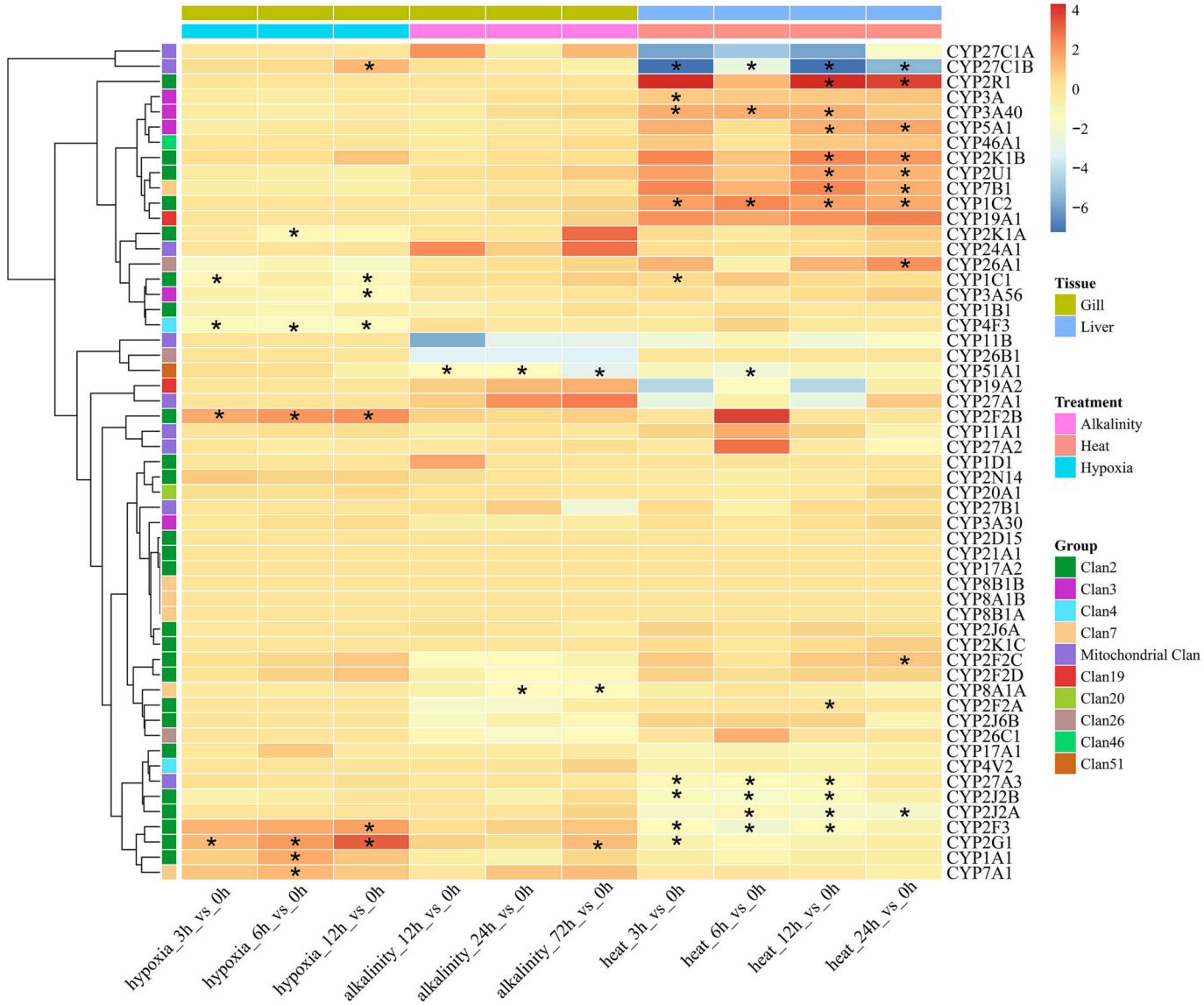

Fig. 6. Heatmap of CYP gene expression in spotted sea bass following experimental challenge with hypoxia, alkalinity and heat from gill and liver tissues. The heatmap is based on log2 (fold change) values. Black * indicates that all values in the experiment reached the threshold (threshold: adjusted *P* value <0.05, total reads ≥5 and log2|fold change| ≥ 1).

significant differential expression for at least one treatment group after stress (Fig. 6). Only one gene was involved in the response to all three tested environmental factor stresses in the gill and liver tissues. Four genes were involved in the response to two of the three tested environmental factor stresses. Twenty-one genes were significantly changed and involved in the response to one of the three tested environmental factor stress.

4. Discussion

Cytochrome P450 (CYP) is a superfamily of enzymes usually known for their vital role in the oxidative conversion of endogenous and exogenous molecules (Nelson, 1999; Fischer et al., 2007). Despite their significance, CYP gene families have not been systematically studied in spotted sea bass, a marketable marine teleost along the Chinese coast.

In this study, fifty-five CYP genes were classified into 17 families within 10 clans based on phylogenetic relationships. The syntenic analysis offered additionally orthologous evidence and validated the results of the phylogenetic analysis, which supported the identification and annotation of CYP genes in spotted sea bass. Nevertheless, 19 CYP families have been identified and described in higher vertebrates (Nelson, 2011), and the CYP16 and CYP39 families were missing in spotted sea bass, the phenomenon of which also occurs in the channel catfish (lost the CYP16 and CYP39 families) (Zhang et al., 2014), zebrafish (lost the CYP16 family) (Goldstone et al., 2010) and fugu fish (lost the CYP39 family) (Nelson, 2003; Goldstone et al., 2010). The CYP39 family was previously considered as being unique to mammals which appeared in the tetrapod lineage after divergence from fish, until it was identified in zebrafish (Nelson, 2003; Goldstone et al., 2010). However, CYP39 family was not observed in other fish species. It has been demonstrated that the *CYP39A1* gene (belonging to the CYP39 family) was involved in biochemical reactions for the conversion of cholesterol to bile acids (Goldstone et al., 2010), and with loss of the CYP39 family in most teleost, its function of regulating bile acid metabolism might be replaced by other genes, such as the *CYP7A* gene, which was reported to play functions in promoting the secretion of bile acids in teleost (Dong et al., 2022).

The copy number of CYP genes within each subfamily also varies among species. Compared with mammals, more copy number occurs in several CYP subfamilies in teleost. For example, the cDNA of *CYP1C* has been detected from the spotted sea bass, common carp (*Cyprinus carpio*) (Itakura et al., 2005), scup (*Stenotomus chrysops*) (Godard et al., 2000) and mummichog (*Fundulus heteroclitus*) (Wang et al., 2006). In zebrafish, the *CYP1C* genes (*CYP1C1* and *CYP1C2* genes) was proved to metabolize 17beta-estradiol (E(2)) and benzo[*a*]pyrene (BaP) (Scornaienchi et al., 2010a,b). However, the *CYP1C* genes have not been found in mammals. Similarly, in CYP2K subfamily, *CYP2K*s share a collinearity with human *CYP2W1*, a tumor-specific CYP that oxidizes indoles and chlorazolones (Lee et al., 2018), the enzyme activity of CYP2K have been reported in rainbow trout, zebrafish and Japanese pufferfish (Uno et al., 2012). For example, the *CYP2K1* and *CYP2K6* genes can metabolize lauric acid in rainbow trout and zebrafish respectively, and the *CYP2K1* protein was also induced after exposure to produced formation water (water associated with the oil in the reservoir) in tropical fish (Zhu et al., 2008). Therefore, CYP1C and CYP2K subfamilies may have unique catalytic activity in teleost species. On the contrary, several subfamilies harbored more CYP numbers for mammals compared with teleost species, such as CYP2B and CYP2C subfamilies. Especially CYP2C subfamily in mouse, 15 members have been identified and have important physiological functions, which catalyze the oxidation of arachidonic acid into epoxyeicosatrienoic acids and hydroxyeicosatetraenoic acids (DeLozier et al., 2004). The primary function of the CYP2B subfamily is to mediate the metabolism of barbiturates and some other medications (Omura, 2022). The *CYP2B1* gene in the rat liver and *CYP2B6* gene in human livers are induced by phenobarbital. However, it was not induced by phenobarbital-type inducers in fish (Uno et al., 2012). The *CYP2B* activity was detected in *Channa punctatus* (a freshwater teleost) induced by cypermethrin treatment (Bhutia et al., 2015). Therefore, the gene regulatory system of the CYP2B subfamily that catalyzes exogenous and endogenous compounds may be different between teleosts and mammals.

Research on the function of CYP genes in teleosts is far behind that in mammals. As a representative of the mixed functional oxide enzyme system, several CYP genes that can be involved in metabolites of organophosphorus pesticides and drugs have been reported (Foxenberg et al., 2007; Guengerich, 2008; Ellison et al., 2012; Zanger and Schwab, 2013). For example, CYP members have been characterized in the model species zebrafish (*Danio rerio*), toxicological experiments in zebrafish treated with atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) showed that *CYP3A* genes could be utilized as biomarkers of the occurrence of chemical contaminants in the ecological environment (Dong et al., 2009). In transcriptome sequencing data of Amur stickleback (*Pungitius sinensis*), and the expression patterns under dimethoate treatment demonstrated that most of the CYP genes exhibited differential expression and were involved in the detoxification of dimethoate (Cao and Cheng, 2019). In a study of medaka, the gene expression of *CYP1B* was significantly upregulated after benzo(*a*)pyrene (chemical compound) exposure in comparison with the control group (Willett et al., 2006). To investigate the roles of the CYP gene family in response to trichlorfon exposure in spotted sea bass, qRT–PCR was performed to examine the mRNA expression patterns of 20 CYP genes under the median lethal concentrations (LC_{50}, 48 h). For some CYP genes, knockout in mice indicated that these CYPs are not required for development and physiological homeostasis but rather have altered responses to the toxic and carcinogenic effects of chemicals (Gonzalez and Kimura, 2003). The *CYP1A1* gene, a representative of oxidation metabolism, is the most studied and has been widely used as a detection index for dioxin-like environmental poisons. As shown by our results in spotted sea bass, the expression profile of the *CYP1A1* gene was up-regulated. The same results were also observed in scup (*Stenotomus chrysops*), in which hepatic *CYP1A1* gene mRNA expression was strongly induced at 1 day after injection of 2,3,7,8-tetrachlorodibenzofuran (TCDF) and remained elevated through 14 days (Hahn and Stegeman, 1994). The liver tissue of mangrove killifish (*Kryptolebias marmoratus*) from a 2,3,7,8-tetrachlorodibenzo-p-dioxin-contaminated water had a 2–4-fold increase in the mRNA level of the *CYP1A1* gene when compared with the control group (Haasch et al., 1993). In spotted sea bass, peak expression of the *CYP1A1* gene was reached at 12 h in both the low and high toxicity groups, showing that the *CYP1A1* gene can be used as an environmental biomonitor to respond to trichlorfon. As an organophosphorus pesticide, trichlorfon can cause hepatocellular edema, vacuolization and hyalinisation, which can seriously impair hepatic functions. The disturbance in the hepatic function may be linked to morphological structural changes and the inhibition of some metabolic enzymes essential for maintaining the functional integrity of the liver (Karami-Mohajeri et al., 2017). In addition, trichlorfon-induced DNA damage exhibited a dose-response relationship, which means that the higher the dose, the more serious the injury (Ma et al., 2019). From the results of this study, the relative expression of the low toxicity group was slightly higher than that of the high toxicity group at most time points, which was possibly due to the fact that the high dose of trichlorfon severely disrupted the functional integrity of the liver and metabolic threshold of these CYP genes may get exceeded, resulting in cell apoptosis and a decrease in the expression of CYP gene. Similar results were obtained in large yellow croaker in the liver and kidney tissues, the *cyp450* gene was highly induced by p-Nitrophenol (a type of environmental pollutant) at low concentrations (2 mg/L), and yet its expression gradually decreased with increasing trichlorfon concentrations (8 mg/L) (Kuang et al., 2020). Moreover, it was discovered in the human hepatocytes, in which the mRNA expression of the *CYP3A4* gene was also induced (10–30-fold) in the low concentration group (1 μM), while the levels of induction decreased with higher concentrations (25 μM) (Das

et al., 2006). The relative expression was induced at low concentrations and inhibited at high concentrations, illustrating that spotted sea bass are weakly tolerant to high concentrations of trichlorfon.

The transcriptomic datasets of three common abiotic factors (hypoxia, alkalinity and heat stress) were analyzed to examine the potential response function of CYP genes in spotted sea bass. Under the stress of hypoxia , most of the significantly altered genes were CYP1–4 families genes, especially the *CYP2F2B* and *CYP2G1* genes were noted for significant changes at three time points (3 h, 6 h and 12 h). Interestingly, a large proportion of the CYP1–4 families genes are known to metabolize exogenous substances, which has been confirmed by the results of trichlorfon stress in this study (Fig. 5), indicating the functional diversity of CYP gene in the spotted sea bass. For the stress of alkalinity, the *CYP8A1A* and *CYP51A1* genes were attended to for widespread changes. Stressor-specific expression of *CYP8A1A* and *CYP51A1* genes occurs in the gill tissue under alkalinity in contrast with hypoxic stress. The CYP51 family gene is one of the pivotal enzymes (Dong et al., 2022), involved in lipid metabolism and steroid biosynthesis, which is a sophisticated process that includes electron transfer, the formation of highly reactive iron-oxygen complexes, and the breaking of C—H and other bonds (Guengerich, 2018; Guengerich et al., 2019). It has been reported in the literature that genes related to lipid metabolism (involved in signal transduction) are more predominant in the response and adaptation of fish to alkalinity stress (Zhao et al., 2020). As inhospitable salinity and alkalinity water causes similarly osmotic stress to fish, the relative expression of *cyp51* gene was significantly upregulated in the half-smooth tongue sole (*Cynoglossus semilaevis*) in a low-salt environment (Si et al., 2018), which increased the organic cholesterol synthesis by activating a series of reactions. Therefore, it is reasonable to validate that the *CYP51A1* gene is engaged in the response to alkalinity.

Compared with the gill tissue, more CYP genes of spotted sea bass exhibited significantly differential expression in the liver tissue treated with heat stress, especially at 12 h and 24 h time points, and a total of 19 CYP genes appeared to have significantly different expression. This may be caused by the fact that the liver is the primary metabolic organ and CYP genes are abundant in the microsomal fraction of the liver (Roussel et al., 1998; Manikandan and Nagini, 2018; Esteves et al., 2021). Those CYP genes expression were significantly changed, demonstrating the involvement of CYP genes in adaptation to rapid temperature changes. In the spotted sea bass, the CYP3A subfamily genes (*CYP3A* and *CYP3A40*) showed significant changes. The *CYP3A* gene in teleost is primarily expressed in the liver and intestine tissues (Tseng et al., 2005) and is involved in the biotransformation of xenobiotics (Hegelund and Celander, 2003) and environmental factors (Qiang et al., 2017). About the *CYP3A40* gene, it has been cloned from the medaka (*O. latipes*) (Kullman and Hinton, 2001) and catalyzed 2alpha- and 6beta-hydroxylation (Kashiwada et al., 2005). However, the *CYP3A40* gene acts as an oxidoreductase to bind to heme in the liver of the coral reef fish (*Acanthochromis polyacanthus*) (Chan et al., 2022), implying that there may be species specificity in the function of the *CYP3A40* gene. In contrast with the significantly upregulated genes, the *CYP27C1B* gene exhibited significant downregulation at four time points under heat stress. The *CYP27C1* gene has been shown the function of an enzyme that catalyzes the conversion of vitamin A_1 into vitamin A_2 and played a crucial role in sensory plasticity in zebrafish and sea lamprey (*Petromyzon marinus*) (Enright et al., 2015; Morshedian et al., 2017). Several environmental factors may affect the balance of the conversion of vitamin A_1 into vitamin A_2 including changes in temperature or season (Temple et al., 2006). Knockout of *CYP27C1* gene in zebrafish suppresses the production of vitamin A_2, negates the ability to red-shift its photoreceptor spectral sensitivity, and reduces its ability to observe and respond to near-infrared light (Enright et al., 2015). The downregulation of *CYP27C1B* gene illustrates a decrease in the amount of vitamin A_2, meaning that heat stress may have destabilized the switch from vitamin A_1 to vitamin A_2.

Generally, 26 among 55 CYP genes of spotted sea bass showed significantly differential expression for at least one treatment after environmental stressors in the gill and liver tissues. These results suggest that the CYP gene family may play diverse functions in regulating the abiotic factor stress responses in the spotted sea bass.

5. Conclusions

In summary, we identified 55 CYP genes in spotted sea bass, and their conserved protein motif, phylogenetic tree, chromosomal distribution, copy number and syntenic information were characterized. The qPCR examination results indicated that CYP genes played an essential role in the metabolism of trichlorfon. Moreover, analysis of their expression profiles based on available transcriptome data generated from different environmental stresses supplied potential functional information in response to abiotic stressors of CYP genes in spotted sea bass. This study improved our understanding of the molecular basis of the CYP gene family in response to toxicological and environmental stresses in spotted sea bass and provided primarily clues for future research on their functions in teleosts.

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CRediT authorship contribution statement

Cong Liu: Conceptualization, Methodology, Software and Writing - Original Draft. **Junjie Li:** Visualization, Software. **Xin Qi:** Conceptualization and Resources. **Lingyu Wang:** Methodology and Software. **Donglei Sun:** Data Curation and Software. **Jingru Zhang:** Methodology and Visualization. **Kaiqiang Zhang:** Methodology and Resources. **Jianshuang Li:** Methodology and Software. **Yun Li:** Conceptualization, Funding acquisition, Methodology and Writing - Review & Editing. **Haishen Wen:** Conceptualization, Funding acquisition, Resources and Writing - Review. All authors contributed to the article and approved the submitted version.

Declaration of competing interest

The corresponding authors state that there are no conflicts of interest. There are no other relationships or activities that could appear to have influenced the submitted work.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbd.2023.101078.

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