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Genome-wide identification and characterization of glucose transporter (*glut*) genes in spotted sea bass (*Lateolabrax maculatus*) and their regulated hepatic expression during short-term starvation☆

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ABSTRACT

The glucose transporters (*GLUTs*) are well known for their essential roles in moving the key metabolites, glucose, galactose, fructose and a number of other important substrates in and out of cells. In this study, we identified a total of 21 *glut* genes in spotted sea bass (*Lateolabrax maculatus*) through extensive data mining of existing genomic and transcriptomic databases. *Glut* genes of spotted sea bass were classified into three subfamilies (Class I, Class II and Class III) according to the phylogenetic analysis. *Glut* genes of spotted sea bass were distributed in 15 out of 24 chromosomes. Deduced gene structure analysis including the secondary structure and the three-dimensional structures, as well as the syntenic analysis further supported their annotations and orthologies. Expression profile in healthy tissues indicated that 9 of 21 *glut* genes were expressed in liver of spotted sea bass. During short-term starvation, the mRNA expression levels of 3 *glut* genes (*glut2*, *glut5*, and *glut10*) were significantly up-regulated in liver ($P < 0.05$), indicating their potential roles in sugar transport and consumption. These findings in our study will facilitate the further evolutionary characterization of *glut* genes in fish species and provide a theoretical basis for their functional study.

1. Introduction

Glucose cannot penetrate the lipid bilayer of cells due to its hydrophilicity, thus specific carrier proteins are needed to facilitate its diffusion. Glucose transporters (*GLUT*s) are integral membrane proteins that mediate the transport of monosaccharides, polyols and other small carbon compounds across the membranes of eukaryotic cells (Joost and Thorens, 2001; Thorens and Mueckler, 2009; Augustin, 2010; Mueckler and Thorens, 2013). They are encoded by the *SLC2A* genes, which belong to the major facilitator superfamily (MFS). All GLUT proteins have sequence and structural features in common, in that they possess 12 membrane spanning helices (Doege et al., 2000; Hruz and Mueckler, 2001; Santer et al., 2002; Augustin, 2010). Fourteen GLUT proteins have been identified in humans, and they can be categorized into three subclasses according to sequence similarity and characteristic elements. Class I consists of the five transporter proteins GLUT1-4 and GLUT14. They are characterized by a glutamine in helix 5 and a STSIF-motif in extracellular loop 7 (Hresko et al., 1994; Doege et al., 1998). Class II comprises the four transporters GLUT5, 7, 9, and 11, with the absence of a tryptophan following the conserved GPXXXP motif in helix 10 as their most striking sequence characteristic (Joost and Thorens, 2001). Class III contains the remaining family members GLUT6, 8, 10, 12 and HMIT/GLUT13. The Class III GLUT proteins are structurally distinguishable from the Class I and Class II by a shorter extracellular loop 1 that lacks a glycosylation site, and by the presence of a glycosylation site in loop 9 (Joost and Thorens, 2001). These human transporters can facilitate the movement of small molecule carbohydrates such as glucose or fructose across the membrane and have been implicated other cellular events including the transport of myo-inositol (González-Salgado et al., 2015), uric acid (Itahana et al., 2015), and insulin (Jaldin-Fincati et al., 2017).

Teleosts are generally considered to be glucose intolerant. Although the capacity for using carbohydrates in fish is poor in relation to higher vertebrates (Wilson, 1994), there was circumstantial evidence for

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transport of glucose mediated by *glut* genes (Planas et al., 2000). Thus the analysis of identification, annotation and expression of fish *gluts* would help to clarify the molecular basis of inefficient glycometabolism in fish. Class I *GLUTs* are the most extensively studied glucose transporters and are distinguished by their affinity to glucose, tissue distribution and hormone regulation in all animals. To date, four members of the Class II (*glut1-4*) have been reported in different teleost species. It was reported that in Atlantic cod (*Gadus morhua*), *glut1* was ubiquitous and *glut2* was relatively abundant in tissues that release glucose; the expression of *glut3* was relatively strong in brain, and the mRNA level of *glut4* is relatively high in heart and muscle (Hall et al., 2014). In rainbow trout (*Onchorhynchus mykiss*), expression of *glut2* was found in the liver, kidney and intestine (Krasnov et al., 2001). The studies involving Atlantic cod (*Gadus morhua*) show that *glut3* has ubiquitous tissue distribution, with the most abundant expression in kidney and low levels in brain, eye, gill, heart, liver, and muscle (Hall et al., 2005). In addition, *glut4* mRNA expression has been detected in skeletal muscle in several teleost species including rainbow trout, brown trout (*Salmo trutta*) and tilapia (Wright et al., 1998; Capilla et al., 2002; Díaz et al., 2007).

Compared with the above mentioned *GLUTs*, study on the Class II and Class III family members in teleosts is lacking. According to the studies in mammals, GLUT5 is the first Class II protein to be discovered, and it has a preference for fructose, playing a primary role in mediating the uptake of dietary fructose across the apical membrane of the small intestine (Douard and Ferraris, 2008). Two gene copies of *Glut9*, *Glut9a* and *Glut9b*, have been identified in mice. *Glut9a* is expressed in many tissues including liver, kidney, intestine, chondrocytes and leukocytes, whereas *Glut9b* is only present in the liver and kidney (Keembiyehetty et al., 2006). They are considered urate transporters instead of glucose or fructose transporters (Bibert et al., 2009). *GLUT8* and *GLUT12* are the members of Class III that have received the most attention in mammalian, which were also reported playing a part in sugar transport or utilization (Schmidt et al., 2009; Doege et al., 2000; Carayannopoulos et al., 2000; Rogers et al., 2002; Linden et al., 2006; Waller et al., 2011; Zawacka-Pankau et al., 2011). We noticed that many *GLUT*s in mammals or other higher vertebrates were involved in carbohydrate metabolism, however, the researches about the characteristics and functions of *GLUT*s, especially Class II and Class III in fish species, lag far behind. Spotted sea bass (*Lateolabrax maculatus*) is an economically important marine fish in China, and the production is one of the largest of all cultured marine fish species in the Chinese aquaculture industry (Lee and Yang, 2002; Wen et al., 2016; Wang et al., 2017). To further improve the production of spotted sea bass, various studies have been designed to investigate important economic traits like growth, reproduction and feeding in this species (Wang et al., 2015). Nonetheless, the mechanisms of regulation and utilization of glucose in this species have not been reported. While some fish studies have involved the *glut* gene family (Tseng et al., 2009; H. Li et al., 2018), most of the previous fish studies have focused on single *glut* genes (R. Li et al., 2018; Yang et al., 2017; Balmaceda-Aguilera et al., 2012; Teerijoki et al., 2000).

With the development of high throughout sequencing technology, more fish genomes and transcriptomes have been successfully sequenced. At the same time, gene sequence information about the *glut* family has been annotated, which will provide valuable resources for studies of the gene number, physicochemical properties, structure, and function. In this study, for the first time in aquaculture teleosts, we conducted comprehensive genome-wide identification of *glut* genes, using sequencing databases for spotted sea bass and determined their expression profiles in liver under short-term starvation. Our findings will provide a better understanding of the role of glucose transporters in the liver and the importance of glucose as an energy substrate for fish.

2. Materials and methods

2.1. Ethics statement

All animal experiments were conducted in accordance with the guidelines and approval of the respective Animal Research and Ethics Committees of Ocean University of China (Permit Number: 20141201). The field studies did not involve endangered or protected species.

2.2. Identification and collection of glut gene sequences

To identify *glut* genes in spotted sea bass from the transcriptomic database (NCBI: **SRR4409341** and **SRR4409397**) (Zhang et al., 2017) and the whole genome database (unpublished data), 14 and 19 GLUT amino acid sequences from human and zebrafish, respectively, were used as query sequences for TBLASTN analysis, all with a cutoff E-value of 1e-5. Human and zebrafish sequences were downloaded from NCBI (http://www.ncbi.nlm.nih.gov/) and Ensembl (http://www.ensembl.org). Open reading frames (ORF) of spotted sea bass *gluts* were predicted by ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/) and further verified by Smart-BLAST against the NCBI non-redundant (NR) protein sequence database. All the GLUT protein sequences were analyzed with Pfam scan (http://www.ebi.ac.uk/Tools/pfa/pfamscan/) to confirm the presence of a GLUT-related domain (PF00083.23, Sugar_tr). Comparative analysis of *GLUT* gene family copy number was conducted among several vertebrates whose genome information has been published in NCBI and Ensembl database, including human (GRCh38.p11), mouse (*Mus musculus*) (GRCm38.p6), cattle (*Bos taurus*) (Bos_taurus_UMD_3.1.1), chicken (*Gallus gallus*) (Gallus_gallus-5.0), zebrafish (GRCz11), cavefish (*Astyanax mexicanus*) (Astyanax_mexicanus-2.0), channel catfish (*Ictalurus punctatus*) (IpCoco_1.2), torafugu (*Takifugu rubripes*) (FUGU5) and yellow croaker (*Larimichthys crocea*) (L_crocea_1.0).

2.3. Phylogenetic and syntenic analysis of glut genes

Phylogenetic analysis of *GLUTs* was conducted using amino acid sequences of spotted sea bass combined with several other vertebrate species consisting of human, zebrafish, and cavefish (Supplementary Table S1). Multiple sequence alignment was conducted by MUSCLE (MUltiple Sequence Comparison by Log-Expectation) (Edgar, 2014) with default parameters. The phylogenetic tree was created with MEGA 7.0.26 and inferred using the Maximum Likelihood method (Le and Gascuel, 2008). Initial tree (s) for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the LG + G + F mode. A discrete Gamma distribution was used to calculate differences of model evolutionary rate among sites. All positions containing gaps and missing data were eliminated, and the bootstrapping value was set as 1500 replications (Kumar et al., 2016). The tree was displayed with Interactive Tree Of Life (iTOL, http://itol.embl.de/).

Syntenic analysis was conducted by comparing genomic regions that harbor *glut* genes in spotted sea bass with other vertebrates. The neighboring genes of spotted sea bass *gluts* were identified from the spotted sea bass genome assembly by the Fgenesh program and verified by BLAST against the NCBI non-redundant database. Ensembl and Genomicus database version 91.01 (Louis et al., 2014; Nguyen et al., 2017) were used to determine the conserved syntenic pattern of *GLUT* genes among various vertebrates.

2.4. Gene structure analysis of glut genes

The physicochemical properties and structure of GLUT proteins were analyzed by bioinformatic methods. The chemicophysical properties of predicted GLUT proteins including the number of amino acids, grand average of hydropathicity (GRAVY), molecular weight (MW,

kDa), theoretical isoelectric point (pI), and instability index (II) were calculated by the ProtParam tool (http://web.expasy.org/protparam/). The DNA and cDNA sequences corresponding to each identified *glut* gene from the spotted sea bass genome and transcriptome databases were used to determine the size of exons and the positions of exon-intron boundaries. The exon-intron structure schematic diagrams of *glut* genes were generated using the Gene Structure Display Server (GSDS, http://gsds.cbi.pku.edu.cn/). Motif identification was performed using the Multiple Em for Motif Elicitation program (MEME 4.12.0, http://alternate.meme-suite.org) (Bailey and Elkan, 1994) with the parameters set as follows: the minimal and maximal motif widths were set to 4 and 100 amino acids, respectively, and the number of motifs was 20. Motifs with an E-value < 1e-10 were retained, and other parameters were set to defaults. The images of the motif were constructed using TBtoolsv0.52. The tertiary structures of GLUT proteins were predicted by SWISS-MODEL (https://swissmodel.expasy.org/interactive) and all structure figures were prepared with PyMol2.1.

2.5. Chromosomal location analysis of glut genes

Positional information of *glut* genes was obtained from the annotation information of spotted sea bass genome database. The distribution map of *glut* genes throughout the spotted sea bass genome was protracted using MapDraw V2.1 software (Liu and Meng, 2003).

2.6. Short-term starvation and sample preparation

Before the experiment, 84 healthy spotted sea bass (weights 118.74 ± 0.32 g) were acclimated for 14 days in the indoor cement pool. The water quality indicators are as follows: water temperature 22 ± 1 °C, pH 7.5–7.9, water salinity 27–28‰ and dissolved oxygen (DO) 5 mg/l. All these fish were randomly divided into experimental and control groups and settled in two cement pools (5 m × 5 m × 1 m). After acclimation, the experimental group was fed at 12:30 PM and fasted thereafter, whereas the control group was fed normally. At 0 h, 1 h, 6 h, 12 h, 24 h, 48 and 72 h after fasting, 6 fish were randomly collected from the control and treatment groups and anaesthetized with tricaine methane sulfonate (MS 222, 200 mg/l, 3-aminobenzoic acid ethyl ester, Sigma). Thereafter, fish were sacrificed by decapitation and the livers were quickly dissected, frozen in liquid nitrogen and stored at −80 °C for subsequent RNA extraction, reverse transcription and quantitative real-time RT-PCR (qRT-PCR).

2.7. Expression analysis of glut genes following short-term starvation

Liver samples were homogenized with a mortar-pestle in the presence of liquid nitrogen. The mortars and pestles were baked at 220 °C for 5 h to destroy RNases. Total RNA was extracted using the TRIzol1 reagent (Invitrogen, USA). RNA was treated with the TURBO DNA-free™ kit (Invitrogen) to remove genomic DNA. The concentration and integrity of total RNA were assessed using the Agilent 2100 Bioanalyzer system (Agilent Technologies, USA). The range of RIN values was from 7.7 to 10. First-strand cDNA was synthesized using PrimeScriptTM RT reagent kit (Takara, Otsu, Japan) (Code No. RR037A) following the manufacturer's instructions. Before reverse transcription into cDNA, all RNA concentrations were diluted to 200 ng/μl. The cDNA samples were subsequently used for the determination of *glut* gene expression by qRT-PCR. All gene-specific primers used in qRT-PCR were designed using the Primer 3 software (Premier Biosoft International) and listed in Table 1. The qPCR primer quality testing for *gluts* in spotted sea bass included standard curves, amplification efficiencies and melt curves. Through validation, all melt curves gave single sharp peaks with no evidence of primer dimers. Five dilutions (including stock solution, dilute 10 times, dilute 100 times, dilute 1000 times, and dilute 10,000 times.), three replicates for accuracy of qRT-PCR reactions and the results were shown in the Table 1.

Table 1
Primers used for quantitative RT-PCR in this study.

Primers	Sequences (5′–3′)	Production size (bp)	Amplification efficiencies (%)
18s-F	GGGTCCGAAGCGTTTACT	179	100.9
18s-R	TCACCTCTAGCGGCACAA		
glut1-F	ATTGCGCAGGTGTTTGGAAT	181	98.6
glut1-R	TCTTCAAAACAGCCTTGGCC		
glut2-F	AAAGCATTATGGTCGGTCGC	194	109.0
glut2-R	TCAGGTCTCCCACAAATCCC		
glut3a-F	TTAAACAGGAGGAGCAGGCA	273	109.5
glut3a-R	CGTAGATGGGCTGTTTCACG		
glut3b-F	GCTCCTATGGTGGCGTAGAT	281	104.2
glut3b-R	CAGGAGGAGGAGGCAAGAAA		
glut4-F	CGGTGTGTGACAATTTGGGT	168	103.1
glut4-R	CAACGCCTCAACAGTCCAAA		
glut5-F	GCCCTTCCTCCCTAGTTTGT	179	108.9
glut5-R	CTCCATCACCGTTCATGCAG		
glut6-F	TAACTGGCTGGTGGTCTCTG	182	111.5
glut6-R	TTTTCAGGCCCCGAGAAGAT		
glut8-F	AGTCCCAAGTACCTCACAGC	166	107.1
glut8-R	CTGTGATGAAGACGGCCATG		
glut9l-F	ATCAGAGGTTCCATTGGCCA	214	113.0
glut9l-R	GAAGGGGAGTACACACAGCT		
glut9l1-F	TTTCTTCCTGAGGCTCCCAG	165	103.5
glut9l1-R	CGGACATGGTGATAAGCTGC		
glut10-F	GAGCTGGGCATCATTTCAGG	291	108.1
glut10-R	GAAGATGCAGCAGGACATGG		
glut11a-F	CTTCAATCAGACTGCTCGGC	239	109.4
glut11a-R	AACATTCCGACATACAGCGC		
glut11b-F	ATTGGGATCTTGGCTGGACA	162	104.5
glut11b-R	TCCTTTGTCGATGAGCAGGT		
glut11d-F	CATCCAGGGCTTCATCAACG	155	106.8
glut11d-R	ATGATTTCGCCATCCTGCAC		
glut12-F	AGATTCCGGCAAGTGTCTGA	173	96.6
glut12-R	TCCAAGGGGCTTCATCCATT		
glut13b-F	TTCTTCATGTACACGGGCCT	189	106.0
glut13b-R	GATGTAGTGGACGTTGCGAC		
glut15a-F	CCAACTTCACTGTGGGCTTC	250	109.2
glut15a-R	TCCACACCACCATAGCCATT		
glut15b-F	AAAGGAAGAGTACTGGCCCC	157	104.8
glut15b-R	AGTACGGAACCACTTCAGGG		

To minimize variation among individuals, two individual fish at the same time points were pooled as one sample, and three biological replicates were analyzed for gene expression profiles. qRT-PCR reactions were performed using TaKaRa SYBR® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Code No. RR820B) on a StepOne Plus Real-Time PCR system (Applied Biosystems). The 20 μl qRT-PCR reaction mixture consisted of 2 μl template cDNA, 0.8 μl of each primer (10 μM), 10 μl of SYBR® Premix Ex TaqTM II (Tli RNaseH Plus) (2×), 0.4 μl of ROX Reference Dye (50×) and 6.0 μl of nuclease-free water. All PCR amplifications were performed in 96-well optical plates. The cycling conditions of qRT-PCR were set as follows: denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s. A negative control (their no-template controls) was prepared for each PCR master mix. The expression levels of each gene were calculated using the $2^{-\Delta\Delta CT}$ method, and 18S ribosomal RNA (18S) was used as the reference gene (Wang et al., 2018). The correlation coefficient between the gene expression in the control group and experimental group was determined by SPSS13.0, one-way ANOVA followed by Duncan's multiple range tests and differences were accepted as statistically significant at $P < 0.05$.

3. Results

3.1. Identification of glut genes of spotted sea bass

A total of 21 *glut* genes were identified in spotted sea bass, including *glut1*, *glut1L*, *glut2*, *glut 3a*, *glut3b*, *glut4*, *glut5*, *glut6*, *glut8*, *glut9*, *glut9L*, *glut10*, *glut11a*, *glut11b*, *glut11c*, *glut11d*, *glut12*, *glut13a*, *glut13b*, *glut15a*, and *glut15b*. All these genes possessed a common Pfam motif

Table 2
Characteristics of 21 *glut* genes identified in spotted sea bass.

Gene name	Gene ID	mRNA (bp)	ORF (bp)	Number of amino acids	Molecular weight (Mw)	Theoretical pI	GRAVY	Instability index (II)
glut1	MF405278	4219	1476	491	54,002.54	8.74	0.49	35.65
glut1L	MH329421	1452	1452	483	53,444.73	8.48	0.461	38.33
glut2	MF405279	4077	1482	493	54,043.68	8.16	0.493	43.39
glut3a	MH329422	1596	1596	531	57,117.41	8.49	0.523	46.26
glut3b	MF405280	4599	1551	516	56,301.31	5.83	0.516	41.46
glut4	MF405281	3114	1524	507	55,815	5.93	0.568	43.11
glut5	MF405282	2028	1539	572	62,979.03	8.83	0.483	41.68
glut6	MF405284	2090	1557	518	56,403.44	9.3	0.48	40.08
glut8	MF405285	2164	1488	495	54,071.69	5.44	0.556	37.05
glut9	MH329423	1512	1512	503	51,826.79	7.55	0.492	41.06
glut9L	MF405288	2278	1578	525	57,801.91	6.48	0.479	43.91
glut10	MF405290	2352	1482	493	44,629.06	8.26	0.501	40.75
glut11a	MF405291	2302	1566	521	56,761.01	6.46	0.344	34.49
glut11b	MH329424	1524	1524	507	55,392.22	7.34	0.507	36.7
glut11c	MH329425	1404	1404	467	52,625.39	8.96	0.541	41.88
glut11d	MH329426	1614	1614	537	57,872.37	5.26	0.391	36.71
glut12	MF405293	2751	1743	580	62,061.67	8.28	0.491	34.43
glut13a	MH329427	1968	1968	655	70,758.28	6.71	0.213	44.9
glut13b	MF405294	2532	1827	608	65,569.57	6.46	0.32	47.35
glut15a	MH329428	1569	1569	522	56,946.14	7.57	0.598	37.75
glut15b	MH329429	1365	1365	454	50,222.47	6.98	0.545	41.89

(PF00083.23, Sugar_tr), which is a conserved domain of this sugar transporter family. The number of amino acid residues encoded by spotted sea bass *glut* genes ranged from 417 (*glut10*) to 655 (*glut13a*), and the relative molecular weights (MWs) ranged from 44,629.06 kDa (*glut10*) to 70,758.28 kDa (*glut13a*). The amino-acid composition ratio was basically consistent, except for *glut11b* and *glut11d* (Supplementary Fig. S1). Briefly, the content of serine was higher than threonine in *glut11b*, which was contrary to the results for the other genes. *Glut11d* had a higher content of asparagine than proline, in contrast with the other genes. The predicted pI of *gluts* ranged from 5.26 to 9.3 with 11 *gluts* being > 7.0, and 9 *gluts* < 7.0 (Table 2). Thermal stability analysis based on the instability index (II) indicated that 13 were stable (*glut2*, *glut3a*, *glut3b*, *glut4*, *glut5*, *glut6*, *glut9*, *glut9L*, *glut10*, *glut11c*, *glut13a*, *glut13b*, and *glut15b*) and the other 8 were unstable (*glut1*, *glut1L*, *glut8*, *glut11a*, *glut11b*, *glut11d*, *glut12* and *glut15a*) (Table 2). All sequence information on these genes has been submitted to GenBank and their accession numbers are provided in Table 2.

3.2. Phylogenetic and syntenic analysis of gluts in spotted sea bass

To annotate the *glut* genes in spotted sea bass, a phylogenetic tree was constructed using deduced GLUT amino acid sequences from several fish and higher vertebrates (Fig. 1). The spotted sea bass *gluts* were categorized into three classes (Class I, Class II and Class III; Fig. 1), which was consistent with previous studies (Mueckler and Thorens, 2013), except for the teolest-specific member, *glut15*, as well as two missing mammalian-specific transporters *glut7* and *glut14*. Class I contained six transporters: *glut1*, *glut1L*, *glut2*, *glut3a*, *glut3b* and *glut4*. Class II consisted of the nine members: *glut5*, *glut9*, *glut9L*, *glut11a*, *glut11b*, *glut11c*, *glut11d*, *glut15a* and *glut15b*. Class III comprised 6 family members: *glut6*, *glut8*, *glut10*, *glut12*, *glut13a*, and *glut13b*. Spotted sea bass *gluts* within each class were clustered with their respective counterparts and had the closest relationship with zebrafish and cavefish.

To further confirm the annotation of spotted sea bass *glut* genes, especially for gene members with multiple copies, syntenic analysis was conducted for *glut1*, *glut9*, and *glut11* (Fig. 2). Similar neighboring genes were found between spotted sea bass and zebrafish for *glut1*, *glut1L* (Fig. 2A), *glut9* and *glut9L* (Fig. 2B), suggesting a conserved syntenic relationship for those genes. In detail, *glut1* was located between *padi2*, lrig2, *magi3* and *phc2*; the second *glut1* copy had the similar group of neighboring genes including *smoc1*, *ccdc177*, *plekhd1* and *slc39a9*. The *glut9* gene was close to *drd5*, *otop1*, *tmem128* and *lyar*; and the other copy had a similar region including *adamts9*, *prickle2*, *psmd6*, *atxn7*, and *thoc7*. Based on their identity to zebrafish orthologs, we annotated the spotted sea bass paralogs as "*glut1L*" and "*glut9L*," respectively, following the nomenclature from zebrafish. There were four gene copies (*glut11a*, *glut11b*, *glut11c*, and *glut11d*) for *glut11*, which was the largest *glut* gene group in spotted sea bass. The syntenic analysis supported the annotation of *glut11a* and *glut11b*, which shared similar neighboring genes with zebrafish *glut11a* and *glut11b* (Fig. 2C). Conserved synteny was found between spotted sea bass *glut11c* and zebrafish *glutl1L* (Fig. 2C), in that *glut11c* was located between *gnaz*, *nt5c2l1*, *kcnn2*, *drg1* and *stard7*. In addition, spotted sea bass contained one extra copy of *glut11*, named *glut11d*, which shared similar neighbor genes with stickleback, including *vstm4*, *sox9*, *cox19*, *pemt* and *rasd1*. These genes were named based on Trends in Genetics Genetic Nomenclature Guide (Mullins, 1995). In summary, the syntenic analysis provided sufficient evidence for the annotation and nomenclature of the *glut* genes in spotted sea bass.

3.3. Gene copy numbers of gluts

The copy number of *GLUT*-family genes was investigated in spotted sea bass and several other vertebrates including human, mouse, cattle, chicken, zebrafish, cavefish, catfish, fugu, and yellow croaker, and the results were summarized in Table 3. In summary, 21 *glut* genes were identified in spotted sea bass, which had the largest number of *GLUT* genes, compared with 14 in human, 12 in mouse, 13 in cattle, 15 in chicken, 19 in zebrafish, 16 in cavefish, 21 in catfish, 17 in fugu, and 19 in yellow croaker. In general, the copy numbers of the *GLUT*-family genes were well conserved in mammals, where only one copy of each gene was present, except for two copies of *GLUT9* and *GLUT5* that were found in mouse and cattle, respectively. In chicken, multiple copies were reported for *GLUT5*, *GLUT9* and *GLUT11*. In contrast, multiple copies were identified for several *glut* members in fish species, and the total gene number was significantly higher than other vertebrates. Specifically, (1) *glut1* had multiple copies ranging from 2 to 4 in the fish species we examined; (2) *glut3* had two gene copies in zebrafish and spotted sea bass, whereas the other tested species possessed only one or no copies of this gene; (3) All tested teleosts had 2–5 *glut9* copies except for cavefish; (4) Except cavefish and fugu, multiple *glut11* copies were present in chicken and most of the tested fish species; (5) Only cavefish and spotted sea bass possessed one additional *glut13* copy compared to the other tested organisms; (6) Two *glut15* copies were detected only in

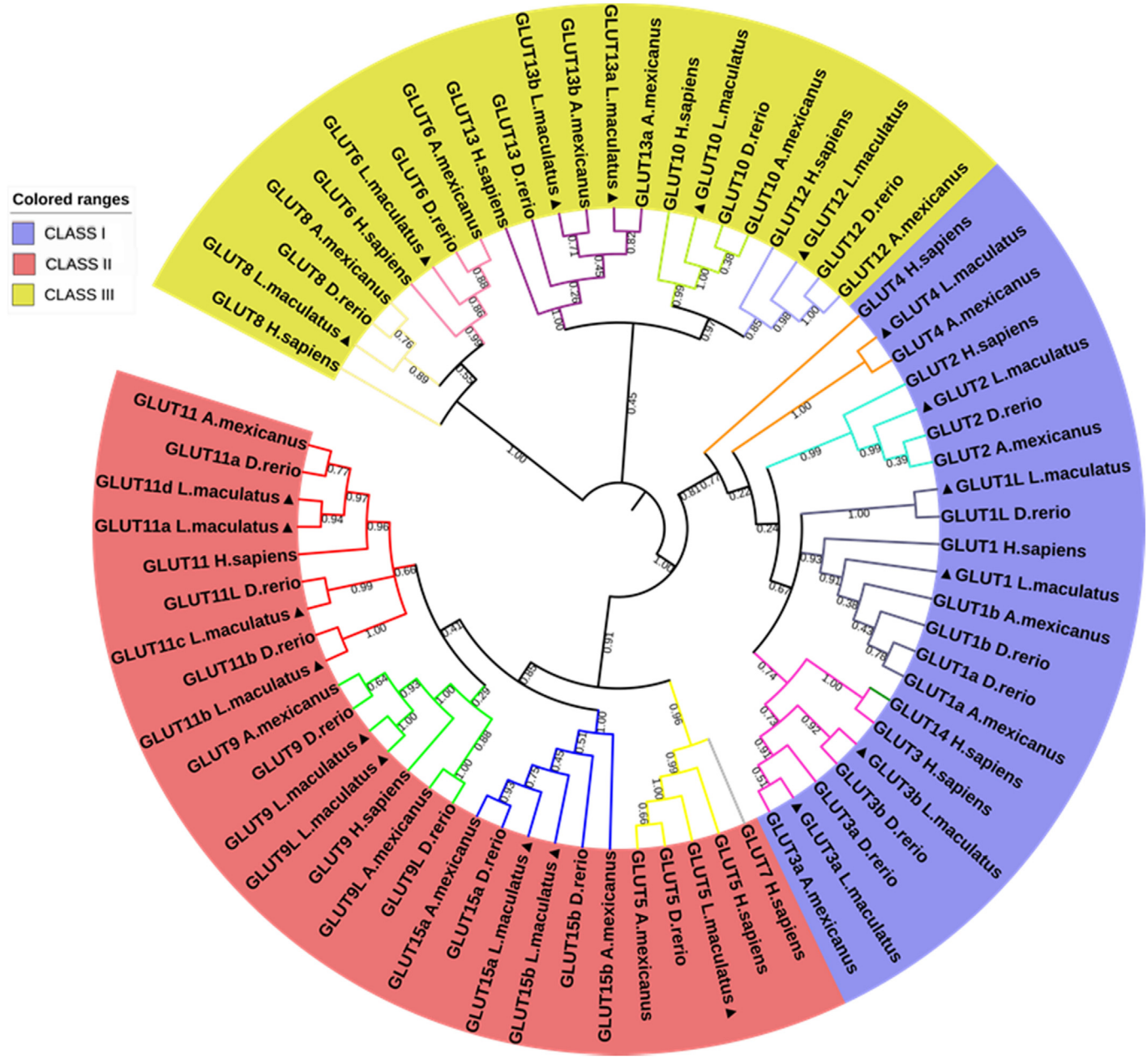

Fig. 1. Phylogenetic tree of *glut* gene family of spotted sea bass. The phylogenetic tree was constructed using the amino acid sequences of *glut* genes from human, zebrafish, cavefish, and spotted sea bass using the method of maximum likelihood under the LG + G + F model by MEGA7 software. The different genes are denoted in different colors of clades. The black triangle indicates spotted sea bass genes. The three color ranges in the figure represent different subfamilies: Class I in red, Class II in blue, and Class III in yellow.

three tested fish species (zebrafish, cavefish, and spotted sea bass); (7) *GLUT7* was found only in human and mouse; and (8) *GLUT14* was a human-specific gene (Table 3).

3.4. Gene structural analysis of glut genes

The number of exons in spotted sea bass *glut* genes varied among different classes. The exon number of *glut* genes in the Class I was consistent which was 10 or 11 (Fig. 3). Most of *glut* genes in Class II contained 11 to 13 exons, except for *glut11a* had 23 exons and *glut11d* had only 1 exon. In Class III, the numbers of exons in *gluts* varied considerably, ranging from 4 to 10; *glut10* and *glut12* genes contained 4 exons, *glut6* had 8 exons, *glut8*, *glut13a* and *glut13b* harbored 10 exons.

To characterize the secondary structure of spotted sea bass *glut* genes, the online tool MEME was used to investigate the possible motifs (Bailey and Elkan, 1994). A total of 20 distinct motifs were found among the *glut* genes (Fig. 4). The results showed that proteins in the same class shared similar motif distribution patterns. The type, order, and number of motifs were similar in Class I and Class II but differed from the proteins in Class III. In general, motifs 1–16 were present in Class I, however, only three proteins (Glut1, Glut3b, and Glut4) had completely coincident motifs 1–15. Other members such as Glut1L lacked motif 8, motif 9, and motif 12, Glut2 lacked motif 1–3 but contains one specific motif 16, and Glut3a lacked the motif 12. Besides conserved motifs 1–15, some specific motifs such as motif16, motif18, and motif19 were included in Class II. Among the proteins in Class II, the motif composition of Glut5 and Glut15a was identical with Glut1, Glut3b and Glut4 in Class I, which possessed all motifs 1–15. Glut11c had the same motif composition as Glut2. Glut9, Glut9L, Glut11a, and Glut11d possessed the specific motif 18, and Glut11a and Glut11d

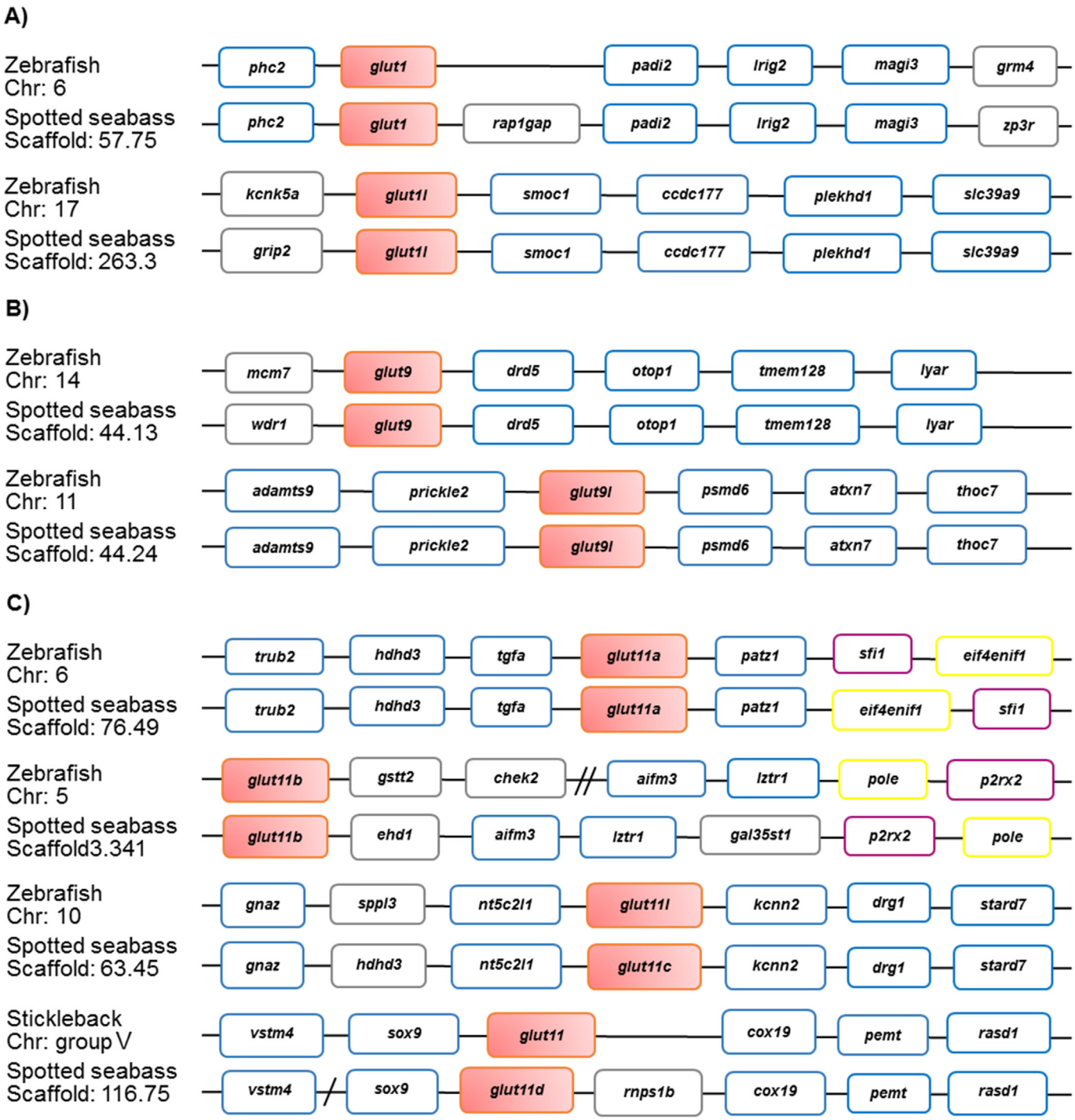

Fig. 2. Syntenic analysis of *glut* genes in selected vertebrates. These syntenies were generated with the information obtained from the genome browser Genomicus based on the existence of neighbor genes. (A) *glut1*, (B) *glut9*, (C) *glut11*. Full gene names are provided in Supplemental Table S2.

contained one specific motif 19. The genes of Class III contained less motif numbers compared with those in Class I and II. All genes in Class III shared common motifs including motif 4, 5, 9, 11, 13, 15, 16, and 17, except for Glut10, which lacked motif 13 and motif 15. Motif 1 existed only in Glut 6, Glut13a and Glut13b. Remarkably, motif 20 was unique to Glut13a and Glut13b.

The prediction of transmembrane domains (TMs) in Gluts from spotted sea bass was based on the predicted protein sequences. The number of TMs in the various family members was different, ranging from 6 to 12 (Fig. 5). Based on the primary and secondary structure analysis of Gluts, tertiary structures were developed by homology modeling methods. A contrastive analysis of the tertiary structures of spotted sea bass Gluts indicated that their three-dimensional structures were conserved, with several exceptions. Such conserved tertiary structures included α helices and random coils, without β sheets. The N and C domains enclosed a cavity that opens to the intracellular side. The tertiary structure of Gluts from Class I consisted of 12 α helices, an extracellular helix and four intracellular helices (IC), and the C-terminal segment was an IC (Fig. 5A). An exception is that the ninth α helix in Glut1L was very short compared with other genes in this class (Fig. 5A). The tertiary structures of Class II members were diverse; most genes comprised 12 α helices, with an IC in the C-terminal region. The

Table 3
Comparison of gene copy numbers of *GLUTs* in selected vertebrates.

Gene	Spotted sea bass	Zebrafish	Channel catfish	Fugu	Cavefish	Yellow croaker	Chicken	Cattle	Mouse	Human
GLUT1	2	3	4	2	2	4	1	1	1	1
GLUT2	1	1	1	1	1	1	1	1	1	1
GLUT3	2	2	1	0	1	1	1	1	1	1
GLUT4	1	0	1	1	1	1	0	1	1	1
GLUT5	1	1	1	4	1	1	2	2	1	1
GLUT6	1	1	1	1	1	1	1	1	1	1
GLUT7	0	0	0	0	0	0	0	0	1	1
GLUT8	1	1	1	1	1	1	1	1	1	1
GLUT9	2	2	5	3	1	3	2	1	2	1
GLUT10	1	1	1	1	1	1	1	1	1	1
GLUT11	4	3	3	1	1	4	4	1	0	1
GLUT12	1	1	1	1	1	1	1	1	1	1
GLUT13	2	1	1	1	2	0	1	1	1	1
GLUT14	0	0	0	0	0	0	0	0	0	1
GLUT15	2	2	0	0	2	0	0	0	0	0
Total	21	19	21	17	16	19	15	13	13	14

number of predicted extracellular helixes varied, some of them having no extracellular helixes, such as Glut5, 11b, and 11d, whereas Glut9 had two small extracellular helixes, and Glut9, 11a, and 11c all had one extracellular helix (Fig. 5B). Specifically, the first transmembrane helix was missing from Glut11d, and only 6 transmembrane helixes and three ICs were predicted for Glut15b (Fig. 5B). Analysis of the tertiary structure of Class III protein suggested that many of them were predicted to contain the characteristic 12 transmembrane helixes, an IC in the C-terminal domain, four ICs, and one extracellular helix (Fig. 5C). Apart from these common features, there were some exceptions, for example, Glut10 lacked many structures like α helix 9, α helix 10, α helix 11, IC5 and IC2. Glut12 did not harbor extracellular helix and IC2, and Glut13b did not have IC1.

3.5. Chromosomal locations of glut genes

The 21 *glut* genes were clearly mapped onto 15 out of 24 chromosomes of spotted sea bass (Fig. 6). The majority of *glut* genes distribute in different chromosomes, for example *glut15b* on chromosome (Chr) 3, *glut13b* on Chr 6, *glut3b* on Chr 7, *glut5* on Chr 9, *glut15a* on Chr 13, *glut6* on Chr 15, *glut3a* on Chr 16, *glut11d* on Chr 17, *glut12* on Chr 18, *glut11c* on Chr 21, *glut13a* on Chr 22 and *glut4* on Chr 23. Chr 4 contained the largest number of *gluts* including *glut1*, *glut10*, *glut9*, *glut9L* and *glut11a*. This was followed by Chr 8 and Chr 19, which contained *glut8* and *glut11b* in the former and *glut1L* and *glut2* in the later.

3.6. Expression of glut genes after short-term starvation

The expression of *glut* genes in liver tissue of spotted sea bass during short-term starvation treatment was examined at 0 h, 1 h, 6 h, 12 h, 24 h, 48 h, and 72 h. Different expression patterns among *glut* genes were observed, reflecting their different regulatory roles. In Class I, the highest expression levels of *glut2* appeared at 48 h and 72 h, when it was

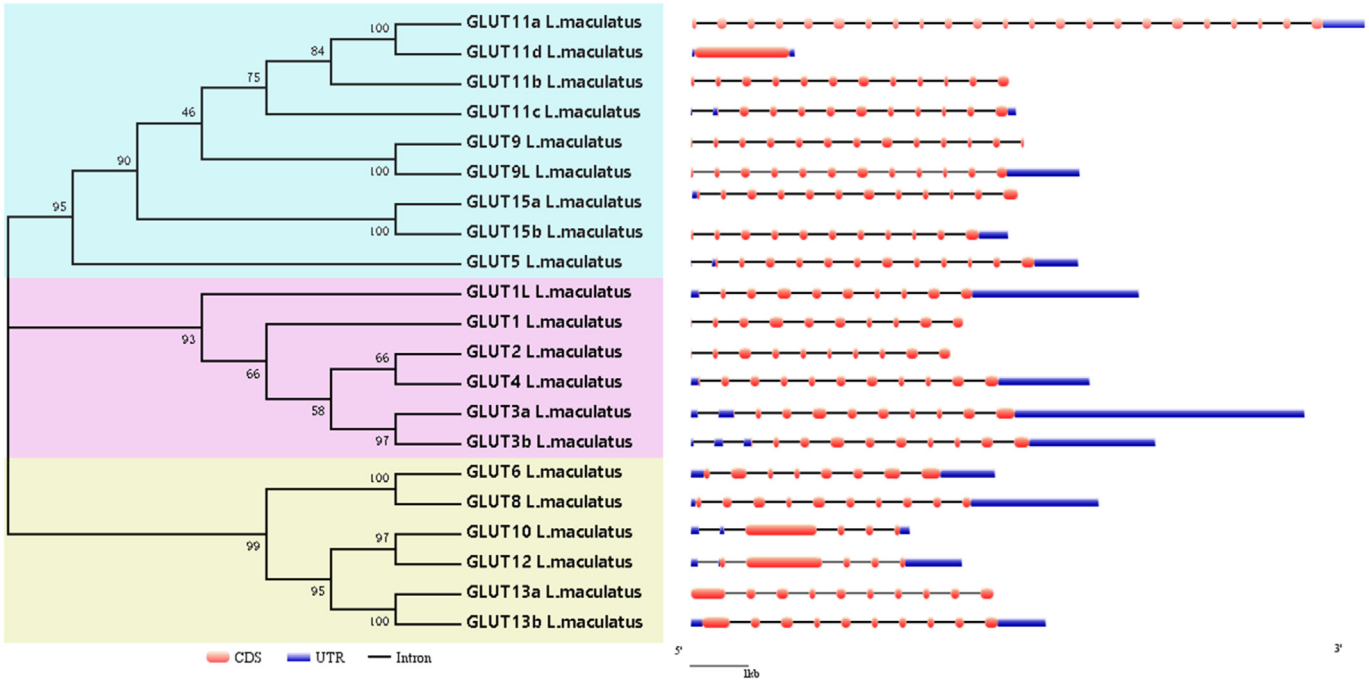

Fig. 3. Exon-intron patterns of the spotted sea bass *glut* genes. The pink boxes, black lines and blue boxes indicate exons, introns and untranslated region (UTR) respectively. The scale in figure is only used for normalizing the lengths of exons. The introns were set as same length follow the instruction of Gene Structure Display Server (GSDS, http://gsds.cbi.pku.edu.cn/). An unrooted tree was constructed based on the full-length amino acid sequences of *glut* genes in spotted sea bass using the method of maximum likelihood under the LG + G + F model with 1000 bootstraps by MEGA7 software. The three subfamilies are indicated by different colors.

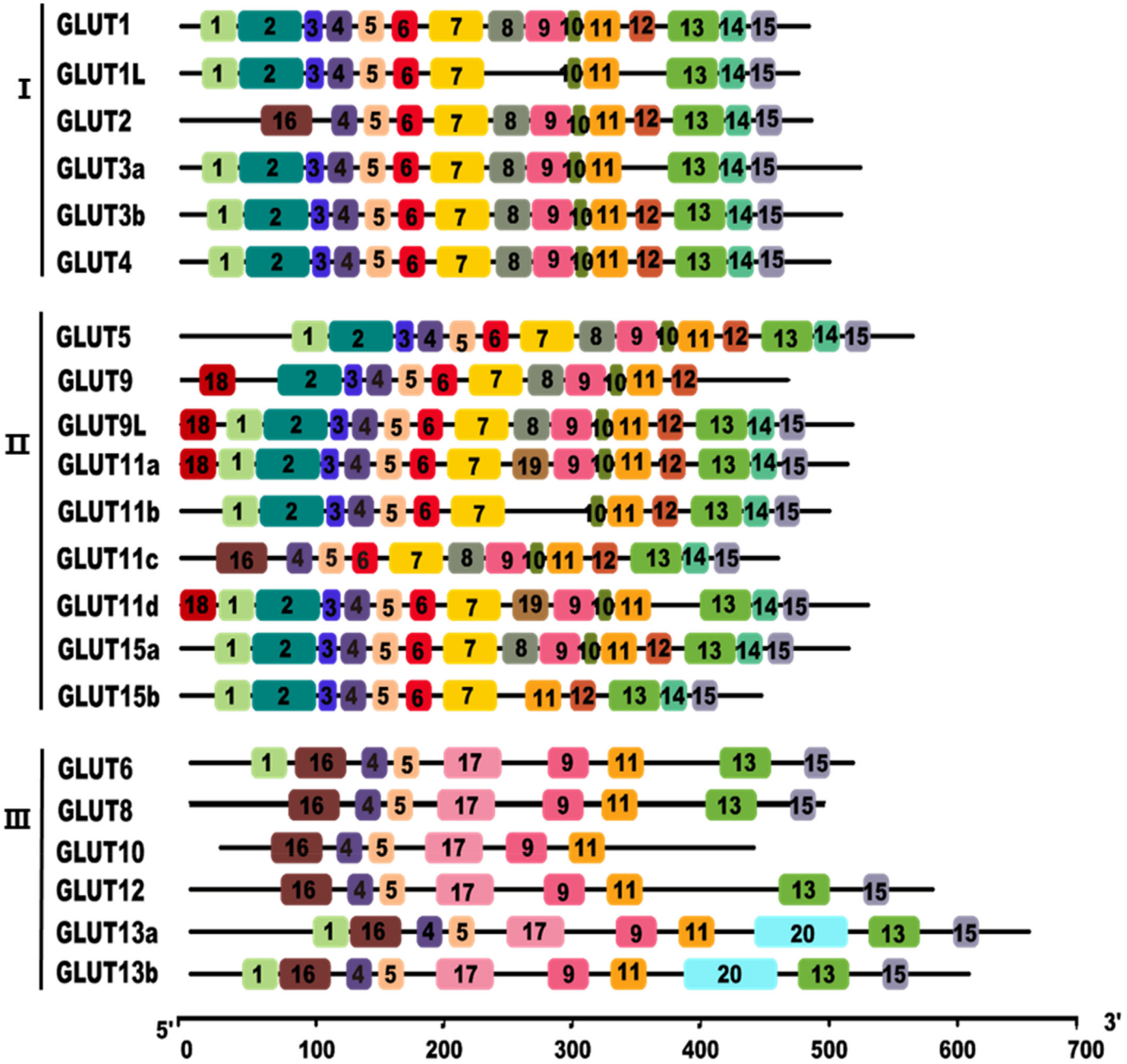

Fig. 4. Distribution of the conserved motifs in *gluts* of spotted sea bass identified by MEME. Black lines represent the non-conserved sequences and colored boxes indicate motif 1 to 20. The bottom line and the numbers indicate the length of GLUT amino acid sequences. The three subfamilies are marked on the left with Roman numerals.

significantly up-regulated > 4-fold compared with its lowest expression level at 12 h ($P < 0.05$) (Fig. 7A). *Glut1* expression did not vary at any time point after starvation except that it was significantly up-regulated at 6 h ($P < 0.05$) (Fig. 7A). The expression of *glut4* was induced at 48 h after starvation, whereas no significant change was detected at any of the other time points ($P < 0.05$) (Fig. 7A). Compared with Class I, the overall expression levels of *glut* genes in Class II were low; only *glut5* was significantly induced by starvation treatment ($P < 0.05$). The highest peak of *glut5* mRNA expression appeared at 24 h then fell gradually at 48 h and 72 h (Fig. 7B). Although expression changes of *glut9* and *glut9L* were detectable, the mRNA quantities of these two *gluts* remained at low levels throughout the experiment (Fig. 7B). In Class III, only *glut10* was regulated by starvation treatment, showing the highest expression level at 6 h and decreasing from 24 h to 72 h (Fig. 7C).

The mRNA expression level of the remaining glut genes, *glut3a* and *glut 3b* in Class I, *glut11a*, *glut11b* and *glut11d* in Class II, *glut8* and *glut13b* in Class III, were almost undetectable (Fig. 7). Taken together, after starvation treatment, *glut* genes were regulated in a gene-specific and time-dependent manner. A total of five *glut* genes were significantly differentially expressed after short-term starvation treatment in spotted sea bass, suggesting their potential involvement in carbohydrate metabolism.

4. Discussion

Glucose transporters are expressed in every cell of the body, and they facilitate cellular uptake of glucose and transfer across the lipid bilayer. Currently, 14 *GLUT* genes have been identified and investigated in humans (Mueckler and Thorens, 2013; Chai et al., 2017), however, only a few studies about the *glut*-family in fish such as largemouth bass

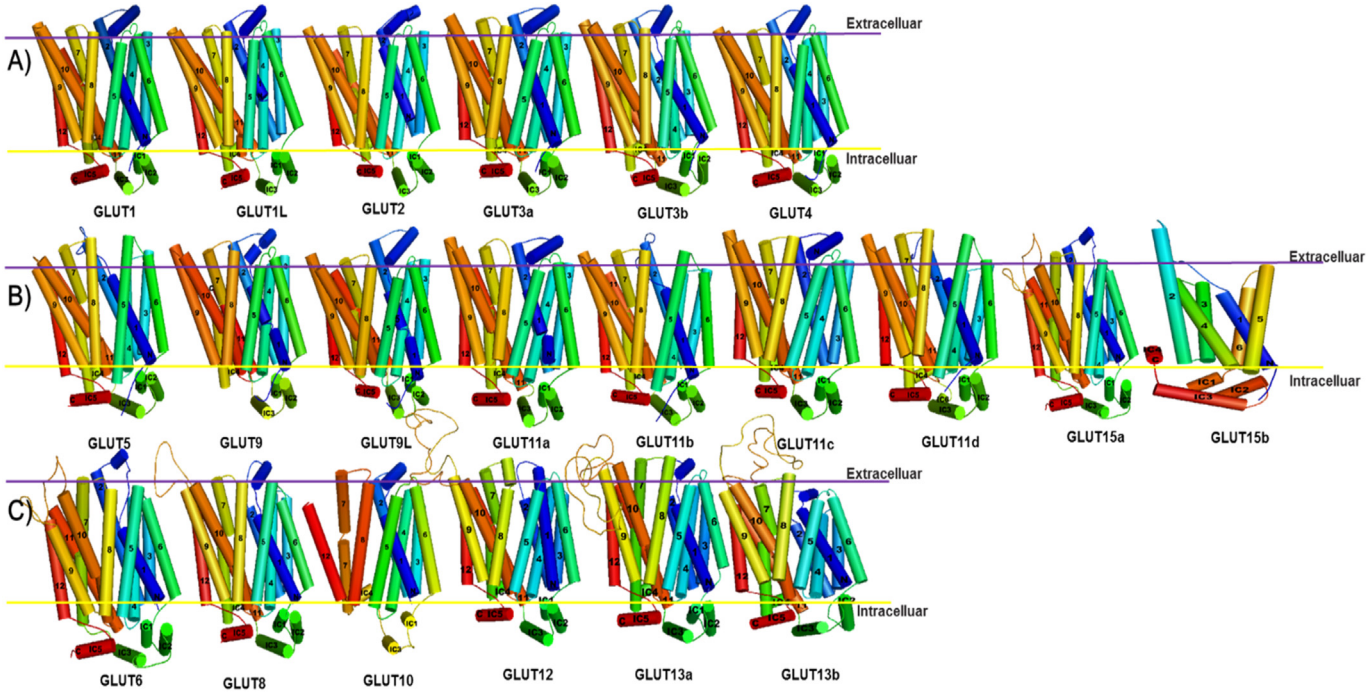

Fig. 5. The tertiary structures of the spotted sea bass deduced GLUTs. A): Class I, B): Class II, C): Class III. The helixes are exhibited in different Arabic numerals. IC indicates intracellular helix. The random coils (coils) are marked as curves. All structure figures were prepared with PyMol.

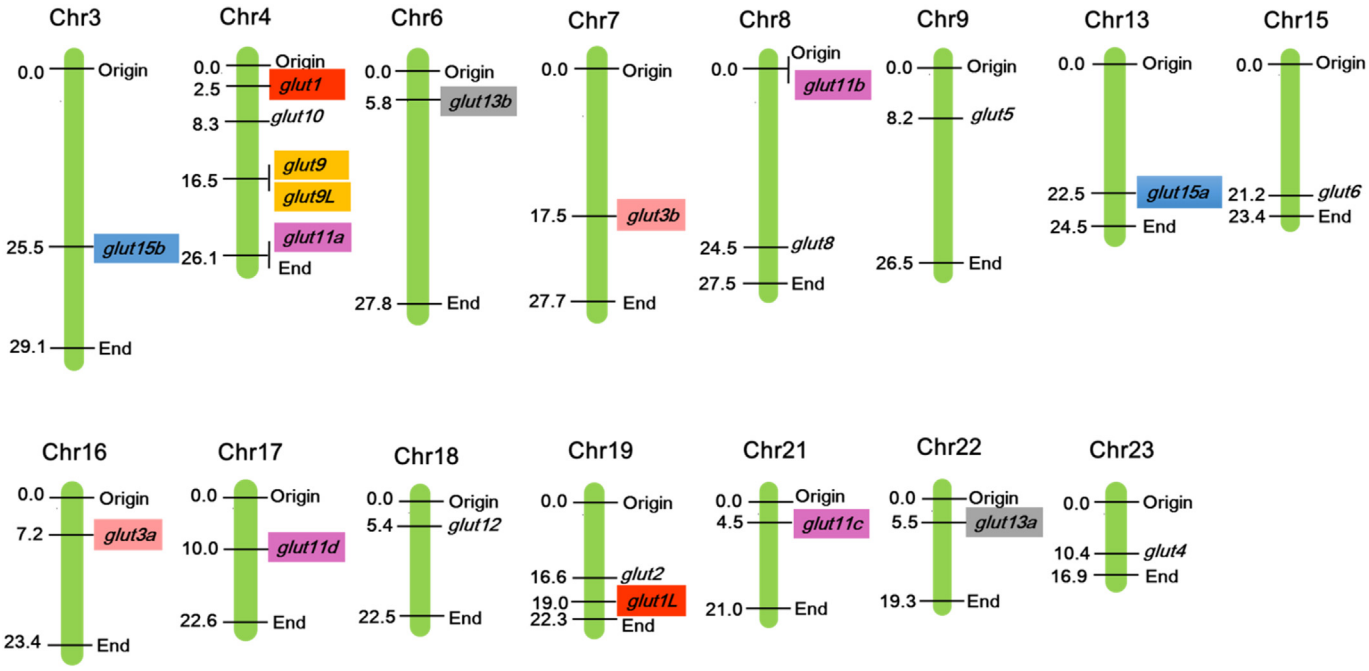

Fig. 6. Distribution of *glut* genes on spotted sea bass chromosomes. Paralogous genes are represented by same colors and single-copy genes are colorless.

(*Micropterus salmoides*) (Yang et al., 2017), pearl gentian grouper (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂) (S. Li et al., 2018), rainbow trout (Teerijoki et al., 2000), and zebrafish (Tseng et al., 2009) have been reported. In this study, 21 *glut* genes were identified and their phylogenetic relationship, intron-exon organization, chromosomal location, conserved motifs, tertiary structures and expression profiles in spotted sea bass liver during starvation were reported. These results should be useful for comparative genomics, especially impact on glucose metabolism studies of fish.

Analysis of physical and chemical properties of *glut* genes in spotted sea bass showed that almost all indicators that we predicted for glut genes were varied in a wide range. The reason for this phenomenon may be space-time specificity, tissue dependence, and various other effects (Kim et al., 2017). The results of Pfam scan indicated that all 21 genes contained the Sugar_tr (PF00083) signature, thus they could potentially encode sugar transporters (Price et al., 2010). The results were identical from higher to lower animals for example human (He et al., 2009), goat (*Capra aegagrus hircus*) (Zhu et al., 2014), Cold Hardiness Frog (*Rana dybowskii*) (Guo et al., 2017), and aphids (*Aphidida*) (Price et al., 2010).

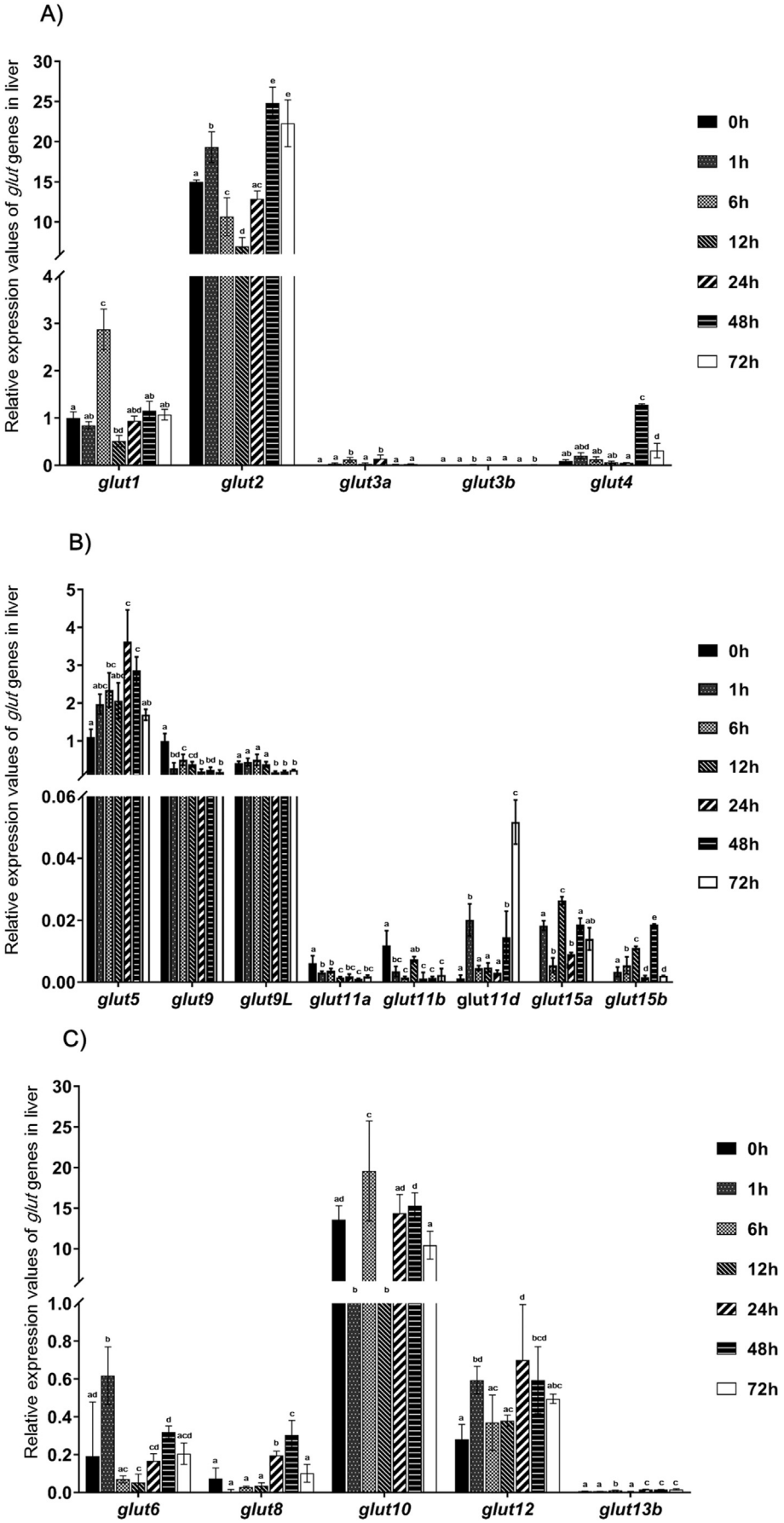

Fig. 7. qRT-PCR analysis for the *glut* genes from (A) Class I, (B) Class II, and (C) Class III in spotted sea bass liver after short-term starvation (0 h–72 h). 18S rRNA was used as an internal control. Significant difference among controls and various treatments performed within different gene group are shown by different letters

The annotations of *glut* genes were dependent upon the phylogenetic and syntenic analysis, which provided simple and intuitive insight to the evolutionary relationship and their identifications (Figs. 1 and 2). Consistent with the classification results of mammal *GLUT* genes based on phylogenetic analyses, *glut* genes of spotted sea bass can be grouped into three similar subfamilies or classes (Uldry and Thorens, 2004). Additionally, we identified fish-specific *glut15*, *glut15a* and *glut15b*, which were grouped in Class II according to phylogenetic analysis.

We also analyzed *glut* gene copy number to further understand the evolution of the *glut* family. Comparison of the copy number of *GLUT* genes in several teloests and respective tetrapods suggests that they are not conserved across species. Teleost species have more *GLUT* gene copies than other vertebrates (Table 3). It is worth mentioning that there are no multiple copies in human (Byers et al., 2017), and only one or two genes are multi-copy in mouse, cattle and chicken, whereas fish have multiple copies of many genes including *glut1*, *glut3*, *glut9*, *glut11*, *glut13* and *glut15*. This result may be caused by teleost-specific whole genome duplication (Jaillon et al., 2004; Berthelot et al., 2014; Glasauer and Neuhauss, 2014). In addition, several species-specific *GLUT*s were observed, including *GLUT7* in human and mouse, *GLUT14* in human, and *glut15* in zebrafish, cavefish, and spotted sea bass, which suggesting either a lineage-specific duplication happened in these species or gene losses occurred in other species (Li et al., 2015).

Based on the gene structural analysis of the *glut* genes in spotted sea bass, we found that the most closely related members in the same class share similar structures and motif composition, and these results were also consistent with the characteristics defined in the phylogenetic analysis (Wilson-O'Brien et al., 2010). *Glut* genes contain 12 hydrophobic transmembrane spanning α-helical domains, have a common Pfam (PF00083.23, Sugar_tr), and 20 other conserved motifs. These results basically match experimental results that had been reported previously (Cura, 2010). Motif analysis indicated that each class had common motifs, and some classes also contained specific motifs (Fig. 5). Intron-exon numbers of *glut* genes in Class I were more conservative than those in Class II and Class III. The majority of the *glut* genes in Class I had 10 exons (Fig. 3), which were consistent with findings in grass carp (*Ctenopharyngodon idellus*) (Zhang et al., 2003), zebrafish (Castillo et al., 2009), fugu (Marín-Juez et al., 2013) and stickleback (*Gasterosteus aculeatus*) (Martínez-Quintana et al., 2014). In Class II, most *glut* genes contained 11–13 exons, and similarly in human, *glut11* and *glut9* contain 11 exons and 12 exons, respectively (Sasaki et al., 2001; Ruiz et al., 2018). It was worth noting that in spotted sea bass, the two duplicated copies of *glut 11*, possessed the largest and smallest number of exons, that *glut*11a harbored 23 exons and *glut11d* contained only 1 exon (Fig. 3). In that case, *glut 11a* of spotted sea bass may gain introns and the *glut 11d* may experience the loss of multiple introns during gene family diversification. In general, systematic comparisons of orthologous eukaryotic genes indicated that many intron positions are conserved over extremely long evolutionary spans (Fedorov et al., 2002; Rogozin et al., 2003). However, the evolutionary gain and loss of introns and exons from genes has been reported and studied in various lineages. Previous studies have reported that an intron-rich gene would lose multiple introns simultaneously by retrotransposition, with the intron-less gene being derived as it must necessarily occur later in evolutionary time (Xie et al., 2008), whereas the great majority of intron gains were associated with the ancient duplications (Babenko et al., 2004). The numbers of exons in *gluts* from Class III varied distinctly, which are consistent with previous studies such as 10 exons were identified in mouse *glut8* (Gawlik et al., 2008; Romero et al., 2009), 5 exons existed in *glut10* of zebrafish (Chiarelli et al., 2011), and 5 exons were characterized in *glut12* of chicken (Coudert et al., 2015).

The tertiary structures of Class I *glut* genes are relatively stable, all consisting of 12 α helices, an extracellular helix and four ICs, and the C-terminal segment was an IC. The tertiary structures of Class II *gluts* are more complex compared with the other groups (Schürmann, 2008). For example *glut15b* gene contains only 6 α helices and four ICs, and no extracellular helix, so its structure is the simplest of the *gluts*; *glut11c* has no first α helix; *glut5*, *glut11b*, and *glut11d* failed to predict the extracellular helix. The results of Class III subfamily tertiary structure analysis showed that they had longer random coils than other class members.

To gain more insight into the roles of spotted sea bass liver in glycometabolism process, the expression profiles of 21 *gluts* under short-term starvation were analyzed by qRT-PCR. There were nine *glut* genes expressed in the liver, but only three of them including *glut2*, *glut5* and *glut10*, had significantly up-regulated expression at tested time point after starvation in spotted sea bass. This observation of genes expressed in the liver is paralleled by findings in other animals, which only a few genes exist in liver and act on glucose transport (Ostrowska et al., 2015; Deng and Yan, 2016). In addition, many studies have reported the expression level of *glut2* and their involvement in glycometabolism. Previous studies have reported in Atlantic cod, the expression of *glut2* in liver decreased during starvation, increased with re-feeding, thus *glut2* was considered to be the reflecting glycogen depletion glucose transport (Hall et al., 2006). The transcription levels of *glut2* in gibel carp (*Carassius auratus gibelio*)starved for 7 d and 21 d were significantly higher than the groups starved for 0 d, 1 d, and 2 d (H. Li et al., 2018). In blunt snout bream (*Megalobrama amblycephala*), *glut2* expression was raised in liver with increasing carbohydrate levels at 3 h after feeding, however, the expression were back to basal at 24 h after feeding, indicating optimal dietary carbohydrate supplementation could enhance the capacity of glucose transfer between liver and blood (Liang et al., 2018). In human, the expression level of *GLUT2* mRNA can affect the capacity of glucose transfer between liver and blood, thereby affects glucose metabolism in the liver (Leibiger and Leibiger, 1995). In the present study, the expression level of *glut2* in spotted sea bass at every tested time point after starvation was significantly higher than the others (Fig. 7A), suggesting its potential involvement in glycometabolism. There are fewer publications on the involvement of *glut5* and *glut10* in glycometabolism compared with *glut2*. We can only generate limited information based on mammals. *Glut5* is the only member specific to fructose transport (Burant et al., 1992; Nomura et al., 2015), and together with *glut2* constitutes the major fructose transporter in the body (Thorens, 2015). In our study *glut5* had lower expression level comparing with the *glut2*, and had rising trends at 0 h–24 h. In rat (*Rattus norvegicus*), the expression of *glut5* gene is subjected to up-regulation by streptozotocin-induced diabetes and starvation (Castello et al., 1995). In our study, the level of spotted sea bass liver *glut10* mRNA have raised in 6 h. Functional analysis has demonstrated that *glut10* transported glucose with relatively high affinity (Dawson et al., 2001), and study revealed the highest expression level of *GLUT10* appeared in the liver of mice (Augustin and Mayoux, 2014). Although studies in several mammalian species have reported the higher expression level of *GLUT5* and *GLUT10* in liver, the involvements of them in carbohydrates consumption and transport, especially in fish species, need further investigation.

In summary, we performed a genome-wide identification of the *glut* genes in spotted sea bass and analyzed their gene structures, phylogenetic relationship, and chromosomal location and expression profiles. We identified a total of 21 *glut* genes, which were unevenly distributed in 15 out of 24 chromosomes. As in higher vertebrates, *glut* genes of spotted sea bass were classified into 3 classes including Class I, Class II and Class III. The expression profiles suggested that *glut* genes of spotted sea bass exhibited time-dependent expression pattern under short-term starvation. Three genes (*glut2*, *glut5* and *glut10*) were highly expressed in the liver and induced by starvation, indicating that these genes may act as functional transporters related to glycometabolism in the liver of spotted sea bass. Overall, our findings contribute to our understanding of *gluts* evolution and shed light on their potential role in glucose transport, metabolism in teleost fish.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbd.2019.03.007.

Conflicts of interest

The authors declare no conflict of interest.

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