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Claudin multigene family in channel catfish and their expression profiles in response to bacterial infection and hypoxia as revealed by meta-analysis of RNA-Seq datasets

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ABSTRACT

Claudins are one of the major groups of transmembrane proteins that play crucial roles in tight junctions. In addition to their function in the regulation of paracellular permeability, claudins are also involved in a number of biological processes related to pathogen infection, embryonic development, organ development and hypoxia response. Despite its importance, analyses of claudin genes in channel catfish have not been systematically performed. In this study, a total of 52 claudin genes were identified and characterized in channel catfish. Phylogenetic analyses were conducted to determine their identities and identify a number of lineage-specific claudin gene duplications in channel catfish. Expression profiles of catfish claudin genes in response to enteric septicemia of catfish (ESC) disease and hypoxia stress were determined by analyzing existing RNA-Seq datasets. Claudin genes were significantly down-regulated in the intestine at 3 h post-infection, indicating that pathogens may disrupt the mucosal barrier by suppressing the expression of claudin genes. A total of six claudin genes were significantly regulated in the gill after hypoxia stress. Among them, the expressions of *cldn-11b* and *cldn-10d* were dramatically altered when comparing hypoxia tolerant fish with intolerant fish, though their specific roles involved in response to hypoxia stress remained unknown.

1. Introduction

Tight junctions are structures that appear in the areas of cell–cell contact where plasma membranes join together. The variability of tight junction tightness makes it possible to form tissue barriers and pores, which can prevent uncontrolled passage of molecules and ions through the intercellular space (Krause et al., 2008). They also function as a fence to separate the apical part from other parts of membrane proteins of epithelial cells. They are involved in the configuration of membrane proteins that are essential for directional transcellular transport (Krause et al., 2008). Four primary groups of tansmembrane proteins are present in epithelial tight junctions: occludin, claudins, the junction-adhesion-molecules (JAMs), and the Coxsackievirus and adenovirus Receptor (CAR) proteins.

Claudins are important for the formation and function of tight junctions (Tsukita and Furuse, 2000). They were first discovered from chicken liver in 1998 as a family of novel integral membrane proteins of tight junctions (Furuse et al., 1998). Since then, many claudin genes have been identified (Kollmar et al., 2001). In mammals, a total of 27 claudin genes have been identified (Mineta et al., 2011; for review see Günzel and Alan, 2013). Among teleosts, 56 claudin genes were identified in Fugu (*Takifugu rubripes*) and at least 43 claudin genes were identified in zebrafish (*Danio rerio*) (Loh et al., 2004; Baltzegar et al., 2013; Kolosov et al., 2013). The number of claudin genes in teleost fish is apparently larger than that in mammals, as a result of fish-specific whole genome duplication in teleosts (Hoegg et al., 2004; Hurley et al., 2007). In contrast to the high numbers of claudin genes in vertebrates, only a few claudin homologues have been identified in invertebrates such as *Drosophila melanogaster* (Wu et al., 2004) and *Caenorhabditis elegans* (Asano et al., 2003).

The primary function of claudins is to regulate the paracellular permeability properties of vertebrate epithelia. Different claudins can either enhance the "barrier" properties of tight junctions or enhance the "leak or pore" forming properties of tight junctions (Kolosov et al., 2013). For example, claudin-2 and claudin-4 were involved in the permeability of intercellular Na^+ and K^+, and claudin-16 is essential for renal resorption of Ca^{2+} and Mg^{2+} (Van Itallie et al., 2001; Müller et al., 2003).

Claudins were also reported to be involved in immune and stress responses. For instance, claudin-1 and claudin-2 were reported to be involved in the early stage of neoplastic transformation, and were up-regulated in the associated inflammatory bowel disease (Weber et al.,

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2008). The barrier formed by claudins and other transmembrane proteins can protect the organisms by preventing the pathogens from penetrating through the epithelial layers. However, some pathogens can affect the structure of tight junctions and influence the synthesis of claudins. Studies on lung diseases revealed that asthma could lead to the fragmentation and thinning of the tight junction belt and down-regulation of claudin genes (Godfrey, 1997). The synthesis and organization of claudins as well as other tight junction proteins could be affected by several integrins and growth factors produced by inflammation (Mazzon and Cuzzocrea, 2007). The down-regulation of claudin-5 in the cells suffering from respiratory distress led to local hypoxia of the lung (Soini, 2011). Furthermore, a recent study reported that the expression of a number of claudins was altered in cancer cells, suggesting their new roles in addition to the regulation of paracellular permeability (Singh et al., 2010). In fish, it has been reported that claudins were involved in responses to growth hormone, cortisol and salinity (Tipsmark et al., 2009; Bui et al., 2010; Clelland et al., 2010; Tipsmark and Madsen, 2012).

With the interest of understanding the claudin genes in the channel catfish genome and their involvement in response to disease infection and hypoxia stress, herein, we report the identification and characterization of 52 claudin genes in the channel catfish (*Ictalurus punctatus*) genome. The identities of catfish claudins were determined by phylogenetic analyses, and their mRNA expression abundance after enteric septicemia of catfish (ESC) disease infection and hypoxia challenges were analyzed by meta-analysis of RNA-Seq datasets, respectively.

2. Materials and methods

2.1. Gene identification and sequence analysis

The initial catfish claudin sequences were identified by searching the channel catfish transcriptome database using TBLASTN, with human, zebrafish (*D. rerio*) and Fugu (*T. rubripes*) claudins as queries. *E-value* was set equal to $1e^{-5}$ to ensure the quality of the results. The transcriptome database was generated by RNA-Seq assembly of a doubled haploid channel catfish (Liu et al., 2012), which has been used as the main resource for the identification of full-length gene transcripts in various catfish gene family studies (Rajendran et al., 2012a, 2012b; Liu et al., 2013a; Wang et al., 2013a, 2014; Zhang et al., 2013; Sun et al., 2014). Duplicates in the initial sequence pool were eliminated by using ClustalW2 (https://www.ebi.ac.uk/Tools/msa/clustalw2/) and a unique set of sequences was subject to further analysis. The unique sequences from the RNA-Seq database were used to blast against catfish draft whole genome sequence database (unpublished) with a cutoff *E-value* of $1e^{-10}$ for further confirmation. Open reading frame (ORF) was identified by using ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The identities of the predicted ORFs were then verified by BLASTP against NCBI non-redundant (nr) protein database with a cutoff *E-value* of $1e^{-5}$. The gene identities were determined by performing phylogenetic analysis with the claudin amino acid sequences from multiple species (see below).

2.2. Phylogenetic analysis

The claudins from several representative vertebrates were selected for phylogenetic analysis, including those from human, mouse and Fugu. The amino acid sequences of claudin genes from these species were retrieved from the NCBI, Ensembl and UniProt databases. Multiple alignment of claudin amino acid sequences was conducted using the ClustalW2 program (Larkin et al., 2007). Phylogenetic analyses were performed using MEGA 5.2 with neighbor-joining method (Tamura et al., 2011). Bootstrapping with 1000 replications was conducted to evaluate the phylogenetic trees, only values greater than 50% were shown.

2.3. Expression of claudin genes in healthy tissues

Meta-analysis was conducted to compare the mRNA expression abundance among healthy tissues, including gill, intestine, liver, ovary and testis. Illumina-based RNA-Seq datasets of control fish (untreated healthy fish) were retrieved from previous and on-going RNA-Seq studies (Li et al., 2012; Sun et al., 2012; Wang et al., 2013b). Normalization was performed for the combined dataset using RUVSeq R package (Risso et al., 2014), which utilized the information of negative controls. Before normalization, a set of in-silico "empirical" negative controls were first identified using edgeR R package (Robinson et al., 2010). These negative control genes were the least significantly differentially expressed genes among the five healthy tissues. The gene read counts of these negative control genes were then used by the RUVg function of the RVUSeq package for claudin gene read count normalization to control the bias in the meta-analysis, such as batch effect, sequencing depth difference and bias generated during sequencing library preparation. After normalization, the mRNA read counts for each of the 52 claudin genes were used to measure the mRNA abundance of each claudin in the five tissues. Claudins with zero mRNA read count were regarded as "undetectable". Claudins with less than five read counts were regarded as "almost undetectable", which were removed in the following ESC disease infection analysis and low oxygen stress response analysis.

2.4. Expression of claudin genes after ESC disease infection

Illumina-based RNA-Seq datasets were retrieved from a previous RNA-Seq study of the entire intestine tissue in catfish after infection with the *Edwardsiella ictaluri* (Li et al., 2012). A detailed protocol of disease challenge, RNA extraction, library construction and sequencing was described in Li et al. (2012). Four time-points (0 h, 3 h, 24 h and 3 d) were included in the analysis. The expression of claudin genes at 3 h, 24 h, and 3 d post-infection was compared with that at 0 h to determine mRNA abundance changes, respectively. Read mapping was performed using CLC Genomics Workbench (version 4.0.2; CLC bio, Aarhus, Denmark). Before mapping, raw reads were trimmed to remove adaptor sequences, ambiguous nucleotides (N's), extremely short reads (<30 bp) and low quality sequences (Quality score < 20). The clean reads from each time-point were then aligned with all gene transcripts assembled previously (Li et al., 2012) in addition with all cDNA sequences of channel catfish claudin genes identified in this study. The mapping parameters were set as: mismatch cost of 2, deletion cost of 3 and insertion cost of 3. The highest scoring matches that shared ≥95% similarity with the reference sequence across ≥90% of their lengths were included in the alignment.

The number of total mapped reads for each claudin gene was determined and normalized to assess the expression level as denoted by RPKM (reads per kilobase of the transcript per million mapped reads). The expression fold change of each claudin gene was calculated based on normalized RPKM. Claudin genes with absolute fold change values ≥ 2, total read number ≥ 5 and p-value ≤ 0.05 were regarded as differentially expressed genes.

2.5. Expression of claudin genes after hypoxia stress

The expressions of claudin genes after hypoxia stress were determined by conducting similar meta-analysis. Illumina-based RNA-Seq datasets were retrieved from our RNA-Seq study in which the gill tissue of catfish after treatment of hypoxia stress was sequenced (NCBI SRA no. SRP039612). Three datasets were used in the study, including sequenced reads from fish tolerant to hypoxia, sequenced reads from fish susceptible to hypoxia, and sequenced reads from control fish without hypoxia stress. The differential expression analyses among these three groups were conducted the same as described above in ESC disease infection.

3. Results

3.1. Identification of channel catfish claudins

A total of 52 claudins were identified from the channel catfish genome, and their sequences have been submitted to GenBank. The accession numbers and sequence characteristics including transcript length, coding sequences, 5′-UTR and 3′-UTR were listed in Table 1. All the 52 claudins encode small proteins with the number of amino acids ranging from 188 to 299; however, their transcripts were relatively long, ranging from 973 bp to 3665 bp with relatively long 5′- and 3′-untranslated regions (UTRs, Table 1). They reside on various chromosomes with chromosome 2 containing the largest number of claudin genes (20 of 52 genes).

Table 1
Summary of 52 claudin genes identified in the channel catfish genome, and characteristics and GenBank accession numbers of their transcripts.

Gene symbol	mRNA (bp)	5′-UTR (bp)	3′-UTR (bp)	CDS (AA)	Chr	Accession
cldn-1	2687	140	1908	213	Chr8	JT417948
cldn-2	1240	275	122	281	Chr13	KM870788
cldn-3a	1442	141	674	209	Chr2	JT349002
cldn-3b	1627	78	892	219	Chr2	JT341870
cldn-3c	1702	807	202	231	Chr13	JT278209
cldn-3d	4018	1095	2260	221	Chr13	KM870801
cldn-5a	1164	407	103	218	Chr11	KM870805
cldn-5c	1929	129	1146	218	Chr2	KM870795
cldn-7a	2249	278	1341	210	Chr25	JT408938
cldn-7b	3770	614	2523	211	Unknown	JT415475
cldn-8b	3231	231	2103	299	Chr22	JT412699
cldn-8c	2895	910	944	347	Chr22	KM870785
cldn-8d	1376	91	391	298	Chr22	JT223127
cldn-10a	1256	323	216	239	Unknown	KM870789
cldn-10b	1889	182	990	239	Unknown	JT413942
cldn-10c	1427	99	569	253	Unknown	JT137346
cldn-10d	1255	129	304	274	Chr12	JT414036
cldn-10e	1266	388	116	254	Chr12	KM870794
cldn-11a	1358	559	145	218	Chr14	JT429221
cldn-11b	3265	198	2413	218	Chr19	JT416620
cldn-11c	1222	419	149	218	Chr22	KM870791
cldn-12	3104	147	1913	348	Chr19	JT405557
cldn-14a	2836	1909	174	251	Chr2	KM870798
cldn-14b	1922	288	887	249	Unknown	JT247091
cldn-14c	2782	201	1798	261	Chr20	JT414263
cldn-15a	1474	281	521	224	Unknown	KM870792
cldn-15b	1366	85	609	224	Chr14	KM870796
cldn-18a	1128	169	245	238	Unknown	JT406165
cldn-18b	2502	298	1442	254	Chr22	KM870797
cldn-19	1533	500	403	210	Chr21	KM870802
cldn-20	3665	82	2851	244	Chr16	JT408105
cldn-23a	2314	237	1264	271	Chr25	JT340271
cldn-23b	2868	566	1612	230	Chr16	KM870791
cldn-25	1443	511	275	219	Chr2	KM870790
cldn-26	1765	881	218	222	Chr22	KM870803
cldn-27a	2362	63	1666	211	Chr2	JT417274
cldn-27b	1042	112	186	248	Chr2	JT407985
cldn-28a	1796	618	539	213	Chr2	KM870804
cldn-28c	2003	100	1339	188	Chr13	JT413728
cldn-29a	1402	52	714	212	Unknown	KM870787
cldn-29b	1479	225	624	210	Chr10	KM870786
cldn-30a	1286	281	360	215	Chr13	JT348620
cldn-30b	973	74	209	230	Chr2	JT412803
cldn-30c	2820	1409	586	275	Chr2	JT412016
cldn-30d	1338	205	203	310	Chr13	KM870806
cldn-31a	1490	268	565	219	Chr10	KM870799
cldn-31b	1123	267	205	217	Chr24	JT411869
cldn-32a	1792	238	918	212	Chr3	JT411066
cldn-32b	4307	1788	1832	229	Unknown	JT407486
cldn-33a	1626	141	816	223	Chr20	KM870800
cldn-33b	2390	193	1528	223	Unknown	JT414960
cldn-33c	1510	257	548	235	Chr26	KM870793

3.2. Phylogenetic analysis

The identities of channel catfish claudins were verified by phylogenetic analysis with the inclusion of claudins from human, mouse, and Fugu. Because a large number of diverse claudin genes were used (154 genes), comprehensive alignment and phylogenetic analysis with all of the sequences were initially conducted to divide the gene members into different clades according to their phylogenetic relationships (Fig. 1). As shown in Fig. 1, a total of ten clades were generated (A to J); separate phylogenetic analysis for each clade was presented in Figs. 2 and 3. Overall, most of the channel catfish claudins were placed into their corresponding clades containing counterparts from other species with strong bootstrap support. A total of 33 channel catfish claudin genes were orthologous to mammalian claudin genes, with the remaining 19 claudins being teleost-specific, which included *cldn-25F*, *-26F*, *-27*, *-28*, *-29*, *-30*, *-31*, *-32*, *-33* and their duplicates.

Clade A consisted of *cldn-3*, *cldn-4* and their duplicates, with a clear evolutionary relationship as shown in Fig. 2A. Clade B consisted of a number of teleost-specific claudin genes including *cldn-27*, *-28*, *-29*, *-30*, and *-32* (Fig. 2B). Clade C contained 15 claudin genes including *cldn-5*, *-6*, *-9* and *-31*. Of which, *cldn-6* and *cldn-9* from human and mouse were grouped together to form a small clade, while Fugu *cldn-6* did not group together with mammalian *cldn-6* (Fig. 2C). Clade D contained 13 claudin genes including *cldn-8*, *-13*, *-17*, and their duplicates. Clade E consisted of *cldn-2*, *-14* and *-20*. Within this clade, a small subclade containing Fugu *cldn-14b*, catfish *cldn-14b* and *cldn-14c* was separated from the subclade containing mammalian *cldn-14* and teleost *cldn-14a* (Fig. 2E). Clade F contained *cldn-1*, *-7* and *-19* with highly resolved phylogenetic relationships (Fig. 3F). Clade G contained *cldn-22*, *-24*, *-25*, and *-33*. The identities of these catfish claudins, *cldn-33a*, *-33b* and *-33c*, were clearly determined (Fig. 3G). Clade H consisted of *cldn-10*, *-15*, *-25* and *-26*. The determination of identities for these five *cldn-10* genes, two *cldn-15* genes, one *cldn-25* gene and one *cldn-26* gene in channel catfish was well-supported by phylogenetic analysis (Fig. 3H). Clade I consisted of *cldn-2* and *cldn-23*, and clade J consisted of *cldn-11*, *-16* and *-18*. Robust phylogenetic relationships were shown in these two clades, providing strong support for determining identities of these claudin genes (Fig. 3I and J).

Phylogenetic analysis revealed specific gene duplications in channel catfish claudins. For instance, *cldn-11* (*cldn-11a* and *cldn-11c*) in clade J, *cldn-10* (*cldn-10a* and *cldn-10b*) in clade H, and *cldn-30* (*cldn-30a* and *cldn-30c*) in clade B were clustered together to form their own clades which were adjacent to respective counterparts (Figs. 2 and 3), suggesting that lineage-specific gene duplication may have occurred in the channel catfish genome during evolution.

3.3. Gene copy number analysis of claudin genes

The copy numbers of claudin genes in channel catfish and other vertebrates such as human, mouse, chicken, Fugu, and zebrafish were determined and summarized in Table 2. In general, only eight claudins (*cldn-4*, *-9*, *-16*, *-17*, *-22*, *-24*, *-25* and *-26*) exist in higher vertebrates (human, mouse, and chicken), and 10 claudins were teleost-specific, including *cldn-25F*, *-26F*, *-27*, *-28*, *-29*, *-30*, *-31*, *-32*, *-33* and *-34*. The copy numbers of claudins in teleost fish are much larger than that of the higher vertebrates. No claudin gene duplications were observed in human, mouse and chicken, but as many as five claudin duplicates were found in teleost species (Table 2). For instance, a total of 52, 56 and 43 claudin genes were identified in the genomes of channel catfish, Fugu and zebrafish, respectively, whereas the total numbers of claudin genes in the genomes of human, mouse, and chicken were 23, 25, and 14, respectively. In channel catfish, 16 claudin genes had more than one copy, 14 of which had multiple copies in the Fugu and zebrafish genomes (Table 2). The duplication of *cldn-18* was only observed in the channel catfish genome, with only one copy of the gene being found in the genomes of Fugu and zebrafish.

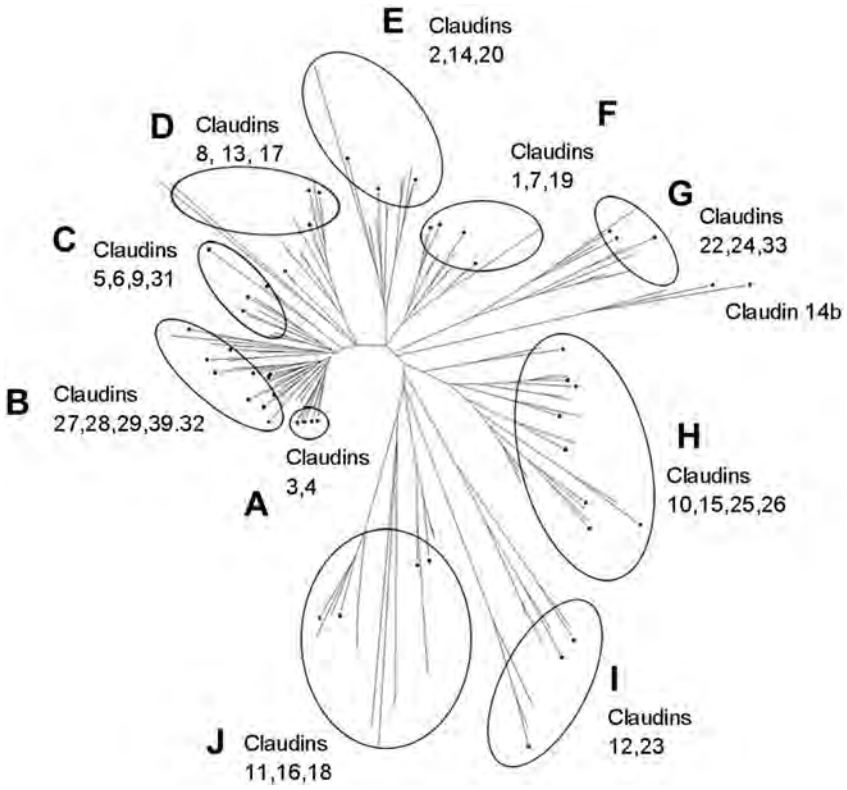

Fig. 1. Consensus phylogenetic tree of all claudin genes. A total of 153 claudin amino acid sequences from human (*Homo sapiens*), mouse (*Mus musculus*), Fugu (*Takifugu rubripes*), and channel catfish (*Ictalurus punctatus*) were used for phylogenetic analysis using neighbor-joining method in MEGA 6. Channel catfish claudins were labeled with black diamond. The tree was divided into ten clades and labeled from clade A to clade J. A detailed phylogenetic tree of each clade was shown in Figs. 2 and 3.

Due to the complexity of the nomenclature of claudin genes, two *cldn-25* (*cldn-25* and *cldn-25F*) and two *cldn-26* (*cldn-26* and *cldn-26F*) were listed in Table 2. In this study, gene symbols *cldn-25* and *cldn-26* represent the corresponding claudins in mammalian species, while *cldn-25F* and *cldn-26F* represent the corresponding claudins in teleost fish. It is notable that *cldn-25* and *cldn-25F* are not orthologs, which is supported by the phylogenetic analysis. Similarly, *cldn-26* and *cldn-26F* are also not orthlogs due to the nomenclature problem in different species.

3.4. mRNA expression abundance of catfish claudins in healthy tissues

The mRNA expression abundance of all the 52 catfish claudin genes in healthy catfish were determined using previous RNA-Seq datasets generated from five tissues, including the gill, intestine, ovary, liver and testis. As shown in Fig. 4, 10 of the 52 claudin genes, including *cldn-1*, *-3c*, *-7a*, *-8d*, *-23a*, *-27b*, *-30a*, *-30c*, *-32a* and -33b, were expressed at relatively high levels in the gill. In contrast, the expressions of *cldn-2*, *-5c*, *-10a*, *-10b*, *-10c*, *-11c*, *-15b*, *-29a*, *-29b*, *-33a* and *-33c* were undetectable or almost undetectable in the gill. In the catfish intestine, *cldn-14b* was the most abundantly expressed claudin, followed by *cldn-30c* and *-23a* (Fig. 4A and B). In the ovary tissue, *cldn-29a* and *cldn-29b* were extremely abundant, which may be partially caused by the uneven sequence implication during the RNA-Seq library construction. In the liver tissue, *cldn-3b*, *-10d* and *-11b* were three of the highly expressed claudins, while no expression can be detected for 24 catfish claudins, including *cldn-3d*, *-7b*, *-8d*, *-10a*, *-10b*, *-10e*, *-11a*, *-11c*, *-14b*, *-15a*, *-15b*, *-23b*, *-25*, *-27b*, *-28a*, *-29a*, *29b*, *-30b*, *-30d*, *-31a*, *-31b*, *-32a*, *-33a* and *-33c*. In the testis tissue, *cldn-3a* and *cldn-3b* were two of the claudins with high levels of expression, followed by *cldn-10a* and *-30c*. The expressions of a number genes were undetectable or almost undetectable in the testis tissue such as *cldn-8c*, *-8d*, *-10a*, *-10e*, *-15b*, *-18b*, *-23b*, *-27b*, *-28a*, *-28c*, *-30b*, *-30d*, *-31a*, *-31b*, and *-33a*.

3.5. Expression of claudin genes after ESC infection and hypoxia stress

The mRNA expression abundance of claudin genes in response to ESC disease infection were determined in the entire intestine tissue. Four of the 52 claudin genes were significantly down-regulated at 3 h post-infection including *cldn-3a*, *-3b*, *-7a*, and *-20a* (Table 3). *Cldn-3a* and *cldn-5a* were down-regulated at 24 h after ESC challenge, while *cldn-19* and *cldn-33b* were up-regulated. Five of the 52 claudin genes were significantly regulated at 3 d after infection, with three genes (*cldn-3a*, *-3b* and *-20a*) being suppressed and two genes (*cldn-11c* and *cldn-19*) being induced.

The expression of claudin genes under hypoxia conditions was also analyzed. Pairwise comparisons were conducted among groups with different hypoxia tolerances: Tolerant vs Control, Intolerant vs Control, and Tolerant vs Intolerant. Seven of the 52 claudins were significantly regulated in the tolerant group as compared with the control group (Table 4). Among them, five claudins (*cldn-8c*, *-10e*, *-12*, *-31a* and *-32b*) were induced under hypoxia conditions, while two claudins (*cldn-10d* and *cldn-11b*) were suppressed. The comparison between intolerant and control group revealed that only *cldn-3a* of the 52 claudins was down-regulated, while it is notable that the mRNA expression abundance of *cldn-3a* in healthy catfish gill tissue was very low.

The comparison between tolerant group and intolerant group revealed that the mRNA abundance of six claudin genes were significantly altered between the hypoxia-tolerant fish as compared with hypoxia-intolerant fish. Four claudin genes (*cldn-8c*, *-10e*, *-30b* and *-31a*) were expressed significantly higher in tolerant fish than in intolerant fish, and two claudin genes (*cldn-10d* and *-11b*) were expressed higher in intolerant fish than in tolerant fish (Table 4).

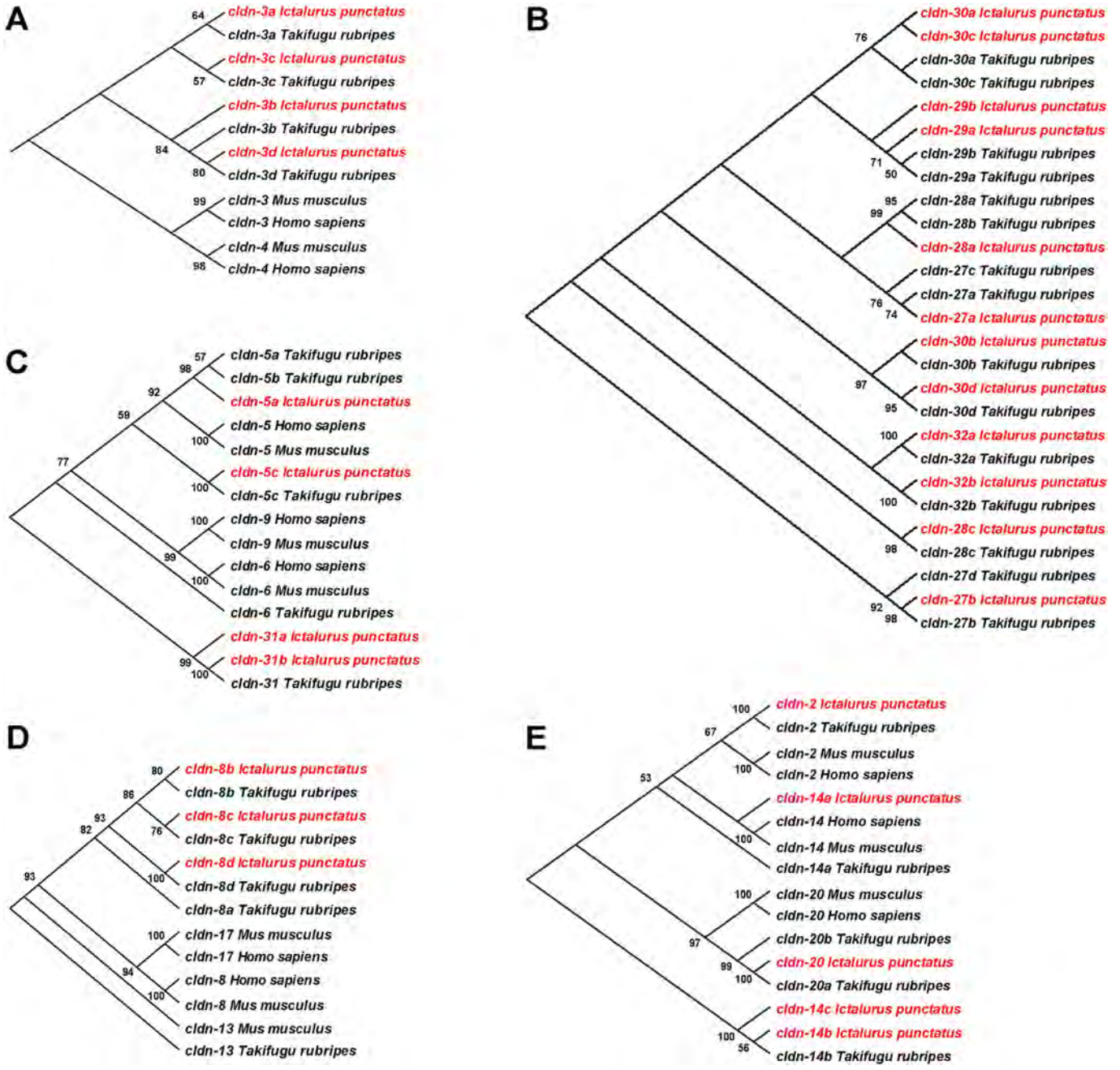

Fig. 2. Detailed phylogenetic trees of claudin clade A to clade E. Claudins of each clade were analyzed separately as described in Fig. 1, with channel catfish claudins being highlighted in red. The bootstrapping values are indicated by numbers at the nodes, shown with only values greater than 50%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Claudins are important functional and structural components of tight junctions, which play critical roles in regulating paracellular permeability (Krause et al., 2008; Kolosov et al., 2013). Here we identified a total of 52 claudin genes in the channel catfish genome, conducted phylogenetic analysis, determined gene copy numbers, and analyzed mRNA expression abundance in response to bacterial infection and hypoxia stress. This is the first systematic work to analyze claudin multigene family in catfish. The provided genomic resources will be useful for functional analysis and comparative genome analysis with other teleost fish. The expansion of claudin multigene family, as observed in the present study, provides a solid model for understanding gene evolution and whole genome duplication among teleost fish.

Annotation of claudins in teleost fishes is complicated. For instance, zebrafish claudins were named after their human orthologs with a numerical designation (e.g., *cldn-1*, *cldn-5*, etc.) or with an alphabetical designation (e.g., *cldn-a*, *cldn-d*, etc.) when they were teleost-specific genes (Kollmar et al., 2001). However, all claudins were named with the numerical designation in Fugu. Genes with human orthologs were assigned with the same names of mammalian counterparts, while the remainders were named sequentially starting from *cldn-25* (Loh et al., 2004). Baltzegar et al. (2013) proposed a revision to the current nomenclature of claudins in zebrafish according to the phylogenetic relationships. In this study, we named the channel catfish claudins after their Fugu and zebrafish orthologs, and validated the identities by conducting phylogenetic analysis. Gene symbols of zebrafish claudins used in this study followed the numerical designation

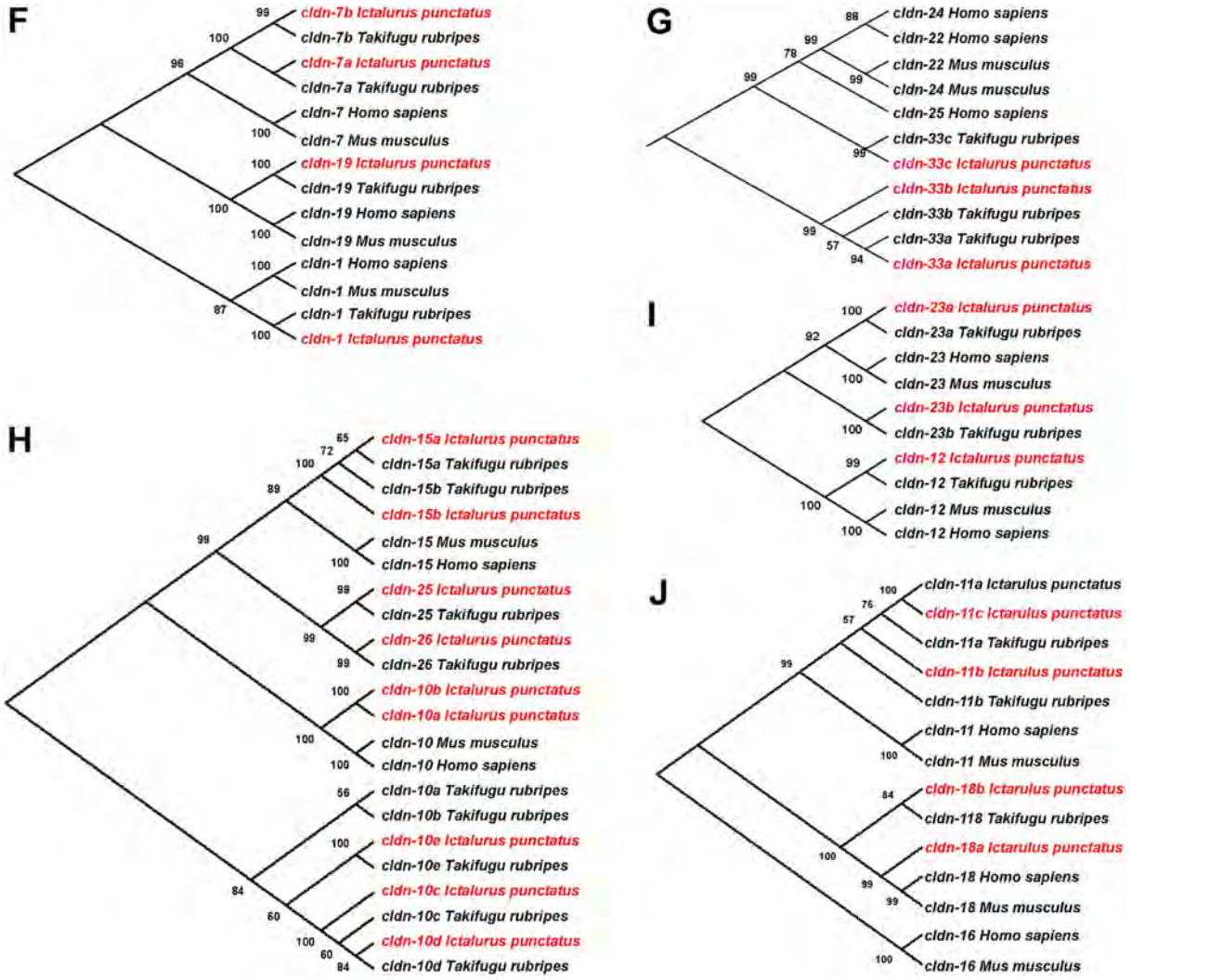

Fig. 3. Detailed phylogenetic trees of claudin clade F to clade J. Claudins of each clade were analyzed separately as described in Fig. 1, with channel catfish claudins being highlighted in red. The bootstrapping values are indicated by numbers at the nodes, shown with only values greater than 50%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

proposed by Baltzegar et al. (2013); Kolosov et al. (2013), for convenience and continuity.

The phylogenetic analysis provided solid support for the identities of channel catfish claudins. As shown in Fig. 1, claudin genes were divided into 10 different clades based on their amino acid sequence similarities, similarly as in the study of zebrafish claudins (Baltzegar et al., 2013). Most of the claudins were clustered with their orthologs in the phylogenetic analysis, while a few exceptions existed. For instance, in clade D, mammalian *cldn-8* was clustered with *cldn-17*, then formed a large clade with teleost *cldn*-8 (Fig. 2D). Syntenic analysis of human claudins indicated that *cldn-8* and *cldn-17* were neighboring genes that were located on human chromosome 21 (data not shown). The high levels of sequence similarity of the two claudins and their genomic co-localization suggested that human *CLDN-8* and *CLDN-17* were derived from tandem duplication in the mammalian lineage. Similarly, lineage-specific duplicated genes were also observed in the channel catfish genome, such as *cldn-11a* and *cldn-11b*, *cldn-10a* and *cldn-10b*, and *cldn-30a* and *cldn-30c*.

The gene copy numbers of claudins are still not finalized in various species, including mammals. After discovery of the first claudin gene in chicken liver, the number of claudin genes was growing gradually. In mammals, 24 claudins were initially discovered (for review see Lal-Nag and Morin, 2009), then three new claudins were recently identified (Mineta et al., 2011). Thus, to date, there are at least 27 claudins in mammalian genomes. In teleost, a total of 63 claudin genes have been reported in 16 teleost species (Kolosov et al., 2013), with 56 claudins being identified in Fugu (Loh et al., 2004) and at least 43 claudins being identified in zebrafish (Baltzegar et al., 2013). In this study, we identified a total of 52 claudins in channel catfish genome with comprehensive genomic resources including several RNA-Seq databases from multiple projects (Liu et al., 2011, 2012; Li et al., 2012; Sun et al., 2012; Wang et al., 2013b), and draft channel catfish whole genome assembly. The vast majority of orthologous genes from both Fugu and zebrafish genome were found in the channel catfish genome (Table 2), suggesting that the most comprehensive set of claudins was identified in channel catfish.

A comparison of gene copy numbers among channel catfish and other representative vertebrate species was conducted to analyze the gene expansion of claudins (Table 2). Notably, only teleost fish contained claudin duplicates, while human, mouse and chicken did not contain any claudin gene duplicates. The fish-specific genome duplication, occurring 226–350 Myr ago (Hurley et al., 2007), was related to the high diversification of teleost fish. As a result of whole genome duplication, teleost fish harbor paralogs that can evolve with partitioned functions or neo-functions to assist adaptation (Hoegg et al., 2004). In this study, a total of 17 claudins possessed multiple copies. Among which, 12 claudins were located on multiple catfish chromosomes and two claudins were located on a single chromosome (Table 1). The

Table 2
Comparison of gene copy numbers of claudin genes among selected vertebrate genomes. Data were based on the Ensembl genome annotation (Release 76), with focus on catfish and vertebrates with sequenced genomes. Fugu and zebrafish claudins were obtained from Baltzegar et al. (2013); Kolosov et al. (2013); Loh et al. (2004). The letter "F" suffixing the *cldn-25F* and *-26F* indicated the *cldn-25* and *-26* genes in teleost fish.

Gene	Human	Mouse	Chicken	Catfish	Fugu	Zebrafish
cldn-1	1	1	1	1	1	1
cldn-2	1	1	1	1	1	1
cldn-3	1	1	1	4	4	4
cldn-4	1	1	0	0	0	0
cldn-5	1	1	1	2	3	2
cldn-6	1	1	0	0	1	0
cldn-7	1	1	0	2	2	2
cldn-8	1	1	1	3	4	3
cldn-9	1	1	0	0	0	0
cldn-10	1	1	1	5	5	4
cldn-11	1	1	1	3	2	2
cldn-12	1	1	1	1	1	1
cldn-13	0	1	0	0	1	0
cldn-14	1	1	1	3	2	0
cldn-15	1	1	0	2	2	3
cldn-16	1	1	1	0	0	0
cldn-17	1	1	0	0	0	0
cldn-18	1	1	1	2	1	1
cldn-19	1	1	1	1	1	1
cldn-20	1	1	1	1	2	0
cldn-21	0	0	0	0	0	0
cldn-22	1	1	0	0	0	0
cldn-23	1	1	0	2	2	2
cldn-24	1	1	0	0	0	0
cldn-25	1	1	1	0	0	0
cldn-26	0	1	0	0	0	0
cldn-25F	0	0	0	1	1	0
cldn-26F	0	0	0	1	1	0
cldn-27	0	0	0	2	4	0
cldn-28	0	0	0	2	3	3
cldn-29	0	0	0	2	2	3
cldn-30	0	0	0	4	4	3
cldn-31	0	0	0	2	1	3
cldn-32	0	0	0	2	2	2
cldn-33	0	0	0	3	3	1
cldn-34	0	0	0	0	0	1

genome locations of the other three claudins remain unknown due to the incompleteness of the catfish genome assembly. The chromosome level distribution of duplicated claudins indicated that many of these duplicates were initially evolved from teleost-specific genome duplication. However, the number of claudin genes in teleost is larger than twice the number of mammalian species, suggesting that whole genome duplication alone is not sufficient to explain the gene copy numbers with claudin genes in teleosts. Apparently, lineage-specific expansion of claudin genes is also involved in the genomes of teleost after the whole genome duplication event, as supported by the presence of claudin genes that are clustered on a single chromosome.

Claudins are involved in responses to pathogen infection and hypoxia stress (Findley and Koval, 2009; Guttman and Finlay, 2009; Gupta and Ryan, 2010; Soini, 2011; Zhang et al., 2012; Kolosov et al., 2013), in addition to its main function as a regulator of paracellular permeability. It is reported that *Clostridium perfringens* enterotoxin (CPE) has increased pathogenicity in the intestine tissue by binding to the C-terminal of certain claudins, including claudin-3, -4, -5, -6, -7, -8, -9 and -14 (Mitchell and Koval, 2010). Altered mRNA expressions of claudin genes, including *cldn-2*, *-3c*, and *-11*, were also observed in cyprinid herpesvirus 3 (CyHV-3) infected common carp (*Cyprinus carpio* L.) in both the upper-mid and lower-mid intestine regions (Syakuri et al., 2013). Another study on CyHV-3 infected common carp demonstrated that the mRNA expression of *cldn-23* and *-30* was significantly down-regulated after CyHV-3 infection in the skin tissue, leading to the disruption of skin barrier and higher susceptibility to secondary infections (Adamek et al., 2013). HIV-1 directly breach the integrity of mucosal epithelial barrier of epithelial cells by decreasing the transcription of *cldn-1*, *-2*, *-3*, *-4* and *-5* and other transmembrane protein genes (Nazli et al., 2010). In the present study, the expressions of claudin genes (*cldn-3a*, *-3b*, *-5a*, *-7a*, *-11c*, *-19*, *-20a* and *33b*) in the entire intestine tissue were significantly down-regulated at the early stages after infection (e.g., 3 h), suggesting that the enteric pathogens disrupted cellular tight junctions to enhance the ability of infection and penetration. It has been reported that the claudin-3 protein isoforms may play a crucial role in hydromineral balance, barrier formation and permeability regulation across intestine epithelium in puffer fish (*Tetraodon nigroviridis*) (Bagherie-Lachidan et al., 2008; Clelland et al., 2010). In Atlantic salmon (*Salmo salar*), *cldn*-3 genes were expressed at high levels in kidney tissue and may play a role in remodeling of the kidney during salinity adaptation (Tipsmark and Madsen, 2012). In rainbow trout, *cldn-5a* and *-33b* mRNAs were relatively abundant in the gill, however, the mRNA abundance of *cldn-5a* and *-33b* was significantly decreased in the gill epithelial cells during the first 3 d of flask culture (Kolosov et al., 2014). After the first 3 d, the mRNA abundance of *cldn-5a* was increased, while the expression of *cldn-33b* was completely suppressed. It is notable that the mRNA expression abundance of claudin genes along the entire intestine tissue was not uniform in fish species (Bagherie-Lachidan et al., 2008; Tipsmark and Madsen, 2012; Syakuri et al., 2013). In this project, the mRNA expression abundance of claudin genes were determined using the entire intestine, rather than the relevant region, which may have underestimated the extent of disease-induced gene regulation.

Studies on hypoxia responses revealed that the expression of claudin genes could be altered in endothelial cells under hypoxia conditions. For instance, hypoxia could disrupt the barrier function of neural blood vessels, and significantly suppress the expression of *cldn-5* (Koto et al., 2007; Ma et al., 2013). Studies on blood–brain barrier (BBB) showed that *cldn*-1 was up-regulated under hypoxia conditions, which had a protective effect against hypoxia-induced increase in blood–brain barrier permeability (Brown et al., 2003). Studies on *cldn-14* showed that mice with heterozygous *cldn-14* displayed abnormal phenotypes including disruption of tight junctions in tumor blood vessels, increased intratumoural leakage and decreased intratumoural level of hypoxia (Baker et al., 2013). Our previous transcriptome profiling of catfish in response to heat stress revealed a few claudin genes that were involved in reorganization of cytoskeleton and extracellular matrix (Liu et al., 2013b), which could play roles in blood–brain barrier permeability.

The expression of catfish claudin genes was determined after acute hypoxia stress in the gill tissue in this study. The gill was selected for analysis because it's the main organ responsible for gas exchange, where oxygen is absorbed into the bloodstream from water. Oxygen-sensitive chemoreceptors contained in the neuroepithelial cells of gill have been shown to sense and initiate hypoxia response, and were responsible for the transition of hypoxic signals through the nervous system to other oxygen-sensitive tissues in the brain, heart, liver, kidney and spleen (Burleson and Smatresh, 1990; Burleson et al,. 2006). As shown in Table 4, *cldn-11b* was the most significantly down-regulated claudin gene among all the 52 claudin genes in the gill tissue of catfish. *Cldn-11b* was expressed in multiple tissues of zebrafish including the gill, kidney, eye, brain, muscle and heart (Clelland and Kelly, 2010; Kumai et al., 2011). It has been reported that cluadin-11 was involved in nerve conduction, consequent hindlimb weakness, spermatocyte differentiation and inner ear deafness (Gow et al., 1999; Kitajiri et al., 2004; Günzel and Alan, 2013). A potential function of claudin-11 is to act as a barrier to blood-derived small molecules (Günzel and Alan, 2013). In this study, *cldn-11b* was down-regulated in tolerant to hypoxia, while no significant expression change was observed in the intolerant catfish (Table 4), indicating that *cldn-11b* was involved in response to low oxygen stress in the gill tissue.

The *cldn-10e* was also down-regulated in catfish under hypoxia conditions, while *cldn-10e* was up-regulated. In puffer fish, it was reported that expression of *cldn-10d* and *-10e* was observed in the gill, skin and

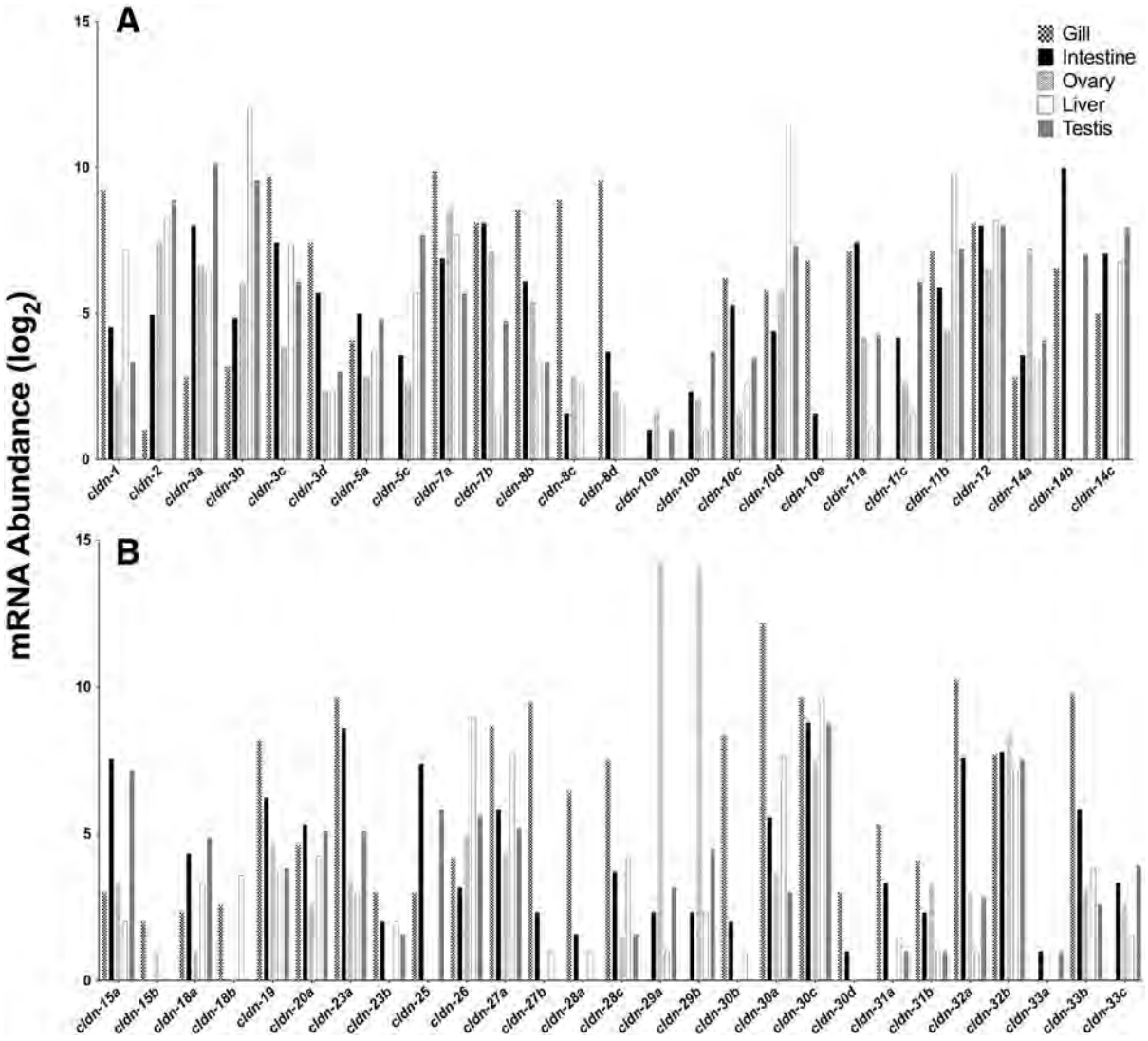

Fig. 4. Expression analysis of 52 catfish claudin genes in healthy catfish. Five tissues were analyzed, including gill, intestine, ovary, liver and testis. The abundance of claudin mRNA was determined using the RNA-Seq gene read counts after normalization by a set of negative controls (see Materials and methods). Y-axis indicated mRNA abundance in read counts after logarithm 2 based transformation.

brain tissue but not in the cultured gill epithelia pavement cell (Bui et al., 2010; Bui and Kelly, 2011). A follow-up study indicated that these claudin genes were present in the gill Na^+-K^+-ATPase immunoreactive ionocytes, suggesting that they may play roles in key changes in the ionomotive activity of the gill epithelium (Bui and Kelly, 2014). Similar as in the puffer fish, *cldn-10d* was mainly expressed in the gill tissue, while it was not found in the flask cultured gill epithelia pavement cells in rainbow trout (Kolosov et al., 2014). In Atlantic salmon, *cldn-10e* was mainly expressed in the gill tissue and its expression was significantly altered after salinity and cortisol stimulation (Tipsmark et al., 2008, 2009).

Phylogenetic analysis of *cldn-10* (Fig. 3, clade H) showed that catfish *cldn-10e* and *cldn-10d* were grouped into different clades, although they all belonged to the large group of *cldn-10*. The differences in amino acids

Table 3
Differentially expressed claudin genes after ESC disease infection. The expressions of all the 52 catfish claudin genes were as determined by meta-analysis of existing RNA-Seq datasets, while only the significantly expressed genes were listed. The significantly expressed genes were identified based on the fold change ≥ |2| with the p-value < 0.05.

Gene	3 h	24 h	3 d
cldn-3a	−2.3	−2.28	−2.91
cldn-3b	−4.71	–	−3.96
cldn-5a	–	−3.31	–
cldn-7a	−3.77	–	–
cldn-11c	–	–	2.52
cldn-19	–	3.63	3.37
cldn-20a	−2.83	–	−2.38
cldn-33b	–	2.12	–

Table 4
Differentially expressed claudin genes after hypoxia stress. The expression of all the 52 catfish claudin genes was determined by meta-analysis of analysis of RNA-Seq datasets. Only the significantly differentially expressed genes are listed (fold change ≥ |2| with the p-value < 0.05).

Gene	Tolerant vs Control	Intolerant vs Control	Tolerant vs Intolerant
cldn-3a	–	−2.08	–
cldn-8c	2.91	–	3.76
cldn-10d	−7.87	–	−6.34
cldn-10e	4.01	–	5.39
cldn-11b	−26.68	–	−26.68
cldn-12	2.10	–	–
cldn-30b	–	–	2.58
cldn-31a	3.08	–	3.08
cldn-32b	2.57	–	–

between catfish *cldn-10d* and *cldn-10e* and their distinct expression pattern under hypoxia conditions suggested that these two claudins could possess distinct functions during evolution.

The differentially expressed claudin genes between the hypoxia-tolerant and intolerant groups are of particular interest. Although the details of their functions are unknown at present, the up-regulated claudins in the tolerant fish could mean that additional expression of these claudin genes may facilitate cellular permeability to enhance tolerance against low oxygen; in contrast, two claudin genes, *cldn-10d* and *cldn-11b*, are drastically lower in tolerant fish than in intolerant fish, suggesting that these claudins may be involved in the tolerance to low oxygen stress.

5. Conclusion

In this study, a total of 52 claudin genes were systematically identified and characterized in the channel catfish genome. Phylogenetic analysis allowed proper annotation of these genes, and provided insights into expansions of claudin gene families in the teleost genomes. The mRNA abundance of claudin genes after disease infection and hypoxia stress were determined. Claudin genes were down-regulated at the early stage after pathogen infection, indicating their potential roles in disrupting mucosal barriers. A number of claudin genes were differentially expressed under hypoxia conditions, suggesting their involvement in response to hypoxia stress. Six claudin genes were differentially expressed between the hypoxia-tolerant and hypoxia-intolerant fish, indicating their significant roles in response to low oxygen stresses.

Conflict of interest

The authors declare no financial or commercial conflict of interest.

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References

Adamek, M., Syakuri, H., Harris, S., Rakus, K.Ł., Brogden, G., Matras, M., Irnazarow, I., Steinhagen, D., 2013. Cyprinid herpesvirus 3 infection disrupts the skin barrier of common carp (*Cyprinus carpio* L.). Vet. Microbiol. 162, 456–470.

Asano, A., Asano, K., Sasaki, H., Furuse, M., Tsukita, S., 2003. Claudins in *Caenorhabditis elegans*: their distribution and barrier function in the epithelium. Curr. Biol. 13, 1042–1046.

Bagherie-Lachidan, M., Wright, S.I., Kelly, S.P., 2008. Claudin-3 tight junction proteins in *Tetraodon nigroviridis*: cloning, tissue-specific expression, and a role in hydromineral balance. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R1638–R1647.

Baker, M., Reynolds, L.E., Robinson, S.D., Lees, D.M., Parsons, M., Elia, G., Hodivala-Dilke, K., 2013. Stromal Claudin14-heterozygosity, but not deletion, increases tumour blood leakage without affecting tumour growth. PLoS ONE 8, e62516.

Baltzegar, D.A., Reading, B.J., Brune, E.S., Borski, R.J., 2013. Phylogenetic revision of the claudin gene family. Mar. Genomics 11, 17–26.

Brown, R.C., Mark, K.S., Egleton, R.D., Huber, J.D., Burroughs, A.R., Davis, T.P., 2003. Protection against hypoxia-induced increase in blood–brain barrier permeability: role of tight junction proteins and NFκB. J. Cell Sci. 116, 693–700.

Bui, P., Kelly, S.P., 2011. Claudins in a Primary Cultured Puffer Fish (*Tetraodon nigroviridis*) Gill Epithelium. Claudins. Humana Press, pp. 179–194.

Bui, P., Kelly, S.P., 2014. Claudin-6, -10d and -10e contribute to seawater acclimation in the euryhaline puffer fish *Tetraodon nigroviridis*. J. Exp. Biol. 217 (Pt 10), 1758–1767.

Bui, P., Bagherie-Lachidan, M., Kelly, S.P., 2010. Cortisol differentially alters claudin isoforms in cultured puffer fish gill epithelia. Mol. Cell. Endocrinol. 317, 120–126.

Burleson, M.L., Smatresk, N.J., 1990. Evidence for two oxygen-sensitive chemoreceptor loci in channel catfish, *Ictalurus punctatus*. Physiol. Zool. 63, 208–221.

Burleson, M.L., Mercer, S.E., Wilk-Blaszczak, M.A., 2006. Isolation and characterization of putative O2 chemoreceptor cells from the gills of channel catfish (*Ictalurus punctatus*). Brain Res. 1092, 100–107.

Clelland, E.S., Kelly, S.P., 2010. Tight junction proteins in zebrafish ovarian follicles: stage specific mRNA abundance and response to 17beta-estradiol, human chorionic gonadotropin, and maturation inducing hormone. Gen. Comp. Endocrinol. 168, 388–400.

Clelland, E.S., Bui, P., Bagherie-Lachidan, M., Kelly, S.P., 2010. Spatial and salinity-induced alterations in claudin-3 isoform mRNA along the gastrointestinal tract of the pufferfish *Tetraodon nigroviridis*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 155, 154–163.

Findley, M.K., Koval, M., 2009. Regulation and roles for claudin-family tight junction proteins. IUBMB Life 61, 431–437.

Furuse, M., Fujita, K., Hiiragi, T., Fujimoto, K., Tsukita, S., 1998. Claudin-1 and-2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J. Cell Sci. 141, 1539–1550.

Godfrey, R., 1997. Human airway epithelial tight junctions. Microsc. Res. Tech. 38, 488–499.

Gow, A., Southwood, C.M., Li, J.S., Pariali, M., Riordan, G.P., Brodie, S.E., Dania, J., Bronstein, J.M., Kacha, B., Lazzarini, R.A., 1999. CNS myelin and sertoli cell tight junction strands are absent in Osp/Claudin-11 null mice. Cell 99, 649–659.

Günzel, D., Alan, S.L., 2013. Claudins and the modulation of tight junction permeability. Physiol. Rev. 93, 525–569.

Gupta, I., Ryan, A., 2010. Claudins: unlocking the code to tight junction function during embryogenesis and in disease. Clin. Genet. 77, 314–325.

Guttman, J.A., Finlay, B.B., 2009. Tight junctions as targets of infectious agents. Biochim. Biophys. Acta 1788, 832–841.

Hoegg, S., Brinkmann, H., Taylor, J.S., Meyer, A., 2004. Phylogenetic timing of the fish-specific genome duplication correlates with the diversification of teleost fish. J. Mol. Evol. 59, 190–203.

Hurley, I.A., Mueller, R.L., Dunn, K.A., Schmidt, E.J., Friedman, M., Ho, R.K., Prince, V.E., Yang, Z., Thomas, M.G., Coates, M.I., 2007. A new time-scale for ray-finned fish evolution. Proc. Biol. Sci. 274, 489–498.

Kitajiri, S.I., Furuse, M., Morita, K., Saishin-Kiuchi, Y., Kido, H., Ito, J., Tsukita, S., 2004. Expression patterns of claudins, tight junction adhesion molecules, in the inner ear. Hear. Res. 187, 25–34.

Kollmar, R., Nakamura, S.K., Kappler, J.A., Hudspeth, A., 2001. Expression and phylogeny of claudins in vertebrate primordia. Proc. Natl. Acad. Sci. U. S. A. 98, 10196–10201.

Kolosov, D., Bui, P., Chasiotis, H., Kelly, S.P., 2013. Claudins in teleost fishes. Tissue Barrier e25391.

Kolosov, D., Chasiotis, H., Kelly, S.P., 2014. Tight junction protein gene expression patterns and changes in transcript abundance during development of model fish gill epithelia. J. Exp. Biol. 217, 1667–1681.

Koto, T., Takubo, K., Ishida, S., Shinoda, H., Inoue, M., Tsubota, K., Okada, Y., Ikeda, E., 2007. Hypoxia disrupts the barrier function of neural blood vessels through changes in the expression of claudin-5 in endothelial cells. Am. J. Pathol. 170, 1389–1397.

Krause, G., Winkler, L., Mueller, S.L., Haseloff, R.F., Piontek, J., Blasig, I.E., 2008. Structure and function of claudins. Biochim. Biophys. Acta 1778, 631–645.

Kumai, Y., Bahubeshi, A., Steele, S., Perry, S.F., 2011. Strategies for maintaining Na^+ balance in zebrafish (*Danio rerio*) during prolonged exposure to acidic water. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 160, 52–62.

Lal-Nag, M., Morin, P.J., 2009. The claudins. Genome Biol. 10, 235.

Larkin, M., Blackshields, G., Brown, N., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Li, C., Zhang, Y., Wang, R., Lu, J., Nandi, S., Mohanty, S., Terhune, J., Liu, Z., Peatman, E., 2012. RNA-Seq analysis of mucosal immune responses reveals signatures of intestinal barrier disruption and pathogen entry following *Edwardsiella ictaluri* infection in channel catfish, *Ictalurus punctatus*. Fish Shellfish Immunol. 32, 816–827.

Liu, S., Zhou, Z., Lu, J., Sun, F., Wang, S., Liu, H., Jiang, Y., Kaltenboeck, L., Kucuktas, H., Peatman, E., Liu, Z., 2011. Generation of genome-scale gene-associated SNPs in catfish for the construction of a high-density SNP array. BMC Genomics 12, 53.

Liu, S., Zhang, Y., Zhou, Z., Waldbieser, G., Sun, F., Lu, J., Zhang, J., Jiang, Y., Zhang, H., Wang, X., Rajendran, K.V., Khoo, L., Kucuktas, H., Peatman, E., Liu, Z., 2012. Efficient assembly and annotation of the transcriptome of catfish by RNA-Seq analysis of a doubled haploid homozygote. BMC Genomics 13, 595.

Liu, S., Li, Q., Liu, Z., 2013a. Genome-wide identification, characterization and phylogenetic analysis of 50 catfish ATP-binding cassette (ABC) transporter genes. PLoS ONE 8, e63895.

Liu, S., Wang, X., Sun, F., Zhang, J., Feng, J., Liu, H., Rajendran, K.V., Sun, L., Zhang, Y., Jiang, Y., Peatman, E., Kaltenboeck, L., Kucuktas, H., Liu, Z., 2013b. RNA-Seq reveals expression signatures of genes involved in oxygen transport, protein synthesis, folding, and degradation in response to heat stress in catfish. Physiol. Genomics 45, 462–476.

Loh, Y.H., Christoffels, A., Brenner, S., Hunziker, W., Venkatesh, B., 2004. Extensive expansion of the claudin gene family in the teleost fish, *Fugu rubripes*. Genome Res. 14, 1248–1257.

Ma, X., Zhang, H., Pan, Q., Zhao, Y., Chen, J., Zhao, B., Chen, Y., 2013. Hypoxia/aglycemia-induced endothelial barrier dysfunction and tight junction protein downregulation can be ameliorated by citicoline. PLoS ONE 8, e82604.

Mazzon, E., Cuzzocrea, S., 2007. Role of TNF-alpha in lung tight junction alteration in mouse model of acute lung inflammation. Respir. Res. 8, 1–19.

Mineta, K., Yamamoto, Y., Yamazaki, Y., Tanaka, H., Tada, Y., Saito, K., Tamura, A., Igarashi, M., Endo, T., Takeuchi, K., 2011. Predicted expansion of the claudin multigene family. FEBS Lett. 585, 606–612.

Mitchell, L.A., Koval, M., 2010. Specificity of interaction between *Clostridium perfringens* enterotoxin and claudin-family tight junction proteins. Toxins 2, 1595–1611.
Müller, D., Kausalya, P.J., Claverie-Martin, F., Meij, I.C., Eggert, P., Garcia-Nieto, V., Hunziker, W., 2003. A novel claudin 16 mutation associated with childhood hypercalciuria abolishes binding to ZO-1 and results in lysosomal mistargeting. Am. J. Hum. Genet. 73, 1293–1301.
Nazli, A., Chan, O., Dobson-Belaire, W.N., Ouellet, M., Tremblay, M.J., Gray-Owen, S.D., Arsenault, A.L., Kaushic, C., 2010. Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation. PLoS Pathog. 6, e1000852.
Rajendran, K., Zhang, J., Liu, S., Kucuktas, H., Wang, X., Liu, H., Sha, Z., Terhune, J., Peatman, E., Liu, Z., 2012a. Pathogen recognition receptors in channel catfish: I. Identification, phylogeny and expression of NOD-like receptors. Dev. Comp. Immunol. 37, 77–86.
Rajendran, K., Zhang, J., Liu, S., Peatman, E., Kucuktas, H., Wang, X., Liu, H., Wood, T., Terhune, J., Liu, Z., 2012b. Pathogen recognition receptors in channel catfish: II. Identification, phylogeny and expression of retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs). Dev. Comp. Immunol. 37, 381–389.
Risso, D., Ngai, J., Speed, T.P., Dudoit, S., 2014. Normalization of RNA-Seq data using factor analysis of control genes or samples. Nat. Biotechnol. 32, 896–902.
Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. EdgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140.
Singh, A.B., Sharma, A., Dhawan, P., 2010. Claudin family of proteins and cancer: an overview. J. Oncol. 2010.
Soini, Y., 2011. Claudins in lung diseases. Respir. Res. 12, 10.1186.
Sun, F., Peatman, E., Li, C., Liu, S., Jiang, Y., Zhou, Z., Liu, Z., 2012. Transcriptomic signatures of attachment, NF-κB suppression and IFN stimulation in the catfish gill following columnaris bacterial infection. Dev. Comp. Immunol. 38, 169–180.
Sun, L., Liu, S., Wang, R., Li, C., Zhang, J., Liu, Z., 2014. Pathogen recognition receptors in channel catfish: IV. Identification, phylogeny and expression analysis of peptidoglycan recognition proteins. Dev. Comp. Immunol. 46, 291–299.
Syakuri, H., Adamek, M., Brogden, G., Rakus, K.Ł., Matras, M., Irnazarow, I., Steinhagen, D., 2013. Intestinal barrier of carp (*Cyprinus carpio* L.) during a cyprinid herpesvirus 3-infection: molecular identification and regulation of the mRNA expression of claudin encoding genes. Fish Shellfish Immunol. 34, 305–314.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.
Tipsmark, C.K., Madsen, S.S., 2012. Tricellulin, occludin and claudin-3 expression in salmon intestine and kidney during salinity adaptation. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 162, 378–385.
Tipsmark, C.K., Kiilerich, P., Nilsen, T.O., Ebbesson, L.O., Stefansson, S.O., Madsen, S.S., 2008. Branchial expression patterns of claudin isoforms in Atlantic salmon during seawater acclimation and smoltification. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R1563–R1574.
Tipsmark, C.K., Jørgensen, C., Brande-Lavridsen, N., Engelund, M., Olesen, J.H., Madsen, S.S., 2009. Effects of cortisol, growth hormone and prolactin on gill claudin expression in Atlantic salmon. Gen. Comp. Endocrinol. 163, 270–277.
Tsukita, S., Furuse, M., 2000. Pores in the wall claudins constitute tight junction strands containing aqueous pores. J. Cell Sci. 149, 13–16.
Van Itallie, C., Rahner, C., Anderson, J.M., 2001. Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability. J. Clin. Invest. 107, 1319–1327.
Wang, R., Feng, J., Li, C., Liu, S., Zhang, Y., Liu, Z., 2013a. Four lysozymes (one c-type and three g-type) in catfish are drastically but differentially induced after bacterial infection. Fish Shellfish Immunol. 35, 136–145.
Wang, R., Sun, L., Bao, L., Zhang, J., Jiang, Y., Yao, J., Song, L., Feng, J., Liu, S., Liu, Z., 2013b. Bulk segregant RNA-Seq reveals expression and positional candidate genes and allele-specific expression for disease resistance against enteric septicemia of catfish. BMC Genomics 14, 929.
Wang, R., Zhang, Y., Liu, S., Li, C., Sun, L., Bao, L., Feng, J., Liu, Z., 2014. Analysis of 52 Rab GTPases from channel catfish and their involvement in immune responses after bacterial infections. Dev. Comp. Immunol. 45, 21–34.
Weber, C.R., Nalle, S.C., Tretiakova, M., Rubin, D.T., Turner, J.R., 2008. Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and may contribute to early neoplastic transformation. Lab. Invest. 88, 1110–1120.
Wu, V.M., Schulte, J., Hirschi, A., Tepass, U., Beitel, G.J., 2004. Sinuous is a *Drosophila* claudin required for septate junction organization and epithelial tube size control. J. Cell Biol. 164, 313–323.
Zhang, J., Liss, M., Wolburg, H., Blasig, I.E., Abdelilah-Seyfried, S., 2012. Involvement of claudins in zebrafish brain ventricle morphogenesis. Ann. N. Y. Acad. Sci. 1257, 193–198.
Zhang, J., Liu, S., Rajendran, K., Sun, L., Zhang, Y., Sun, F., Kucuktas, H., Liu, H., Liu, Z., 2013. Pathogen recognition receptors in channel catfish: III Phylogeny and expression analysis of Toll-like receptors. Dev. Comp. Immunol. 40, 185–194.